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# UNITED STATES DEPARTMENT OF AGRICULTURE



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Washington, D. C.



March, 1927

## CITRUS MELANOSE AND ITS CONTROL

By

JOHN R. WINSTON, formerly Associate Pathologist  
JOHN J. BOWMAN and WALTER J. BACH, Junior Pathologists  
Office of Fruit Diseases, Bureau of Plant Industry

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By JOHN R. WINSTON, formerly *Associate Pathologist*, JOHN J. BOWMAN and WALTER J. BACH, *Junior Pathologists*, Office of Fruit Diseases, Bureau of Plant Industry<sup>1</sup>

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#### INTRODUCTION

The purpose of this bulletin is to present the results of investigations on citrus melanose (caused by *Phomopsis citri* Fawcett),<sup>2</sup> to stress the economic importance of the disease, and to assemble information on it by giving a résumé of the more important published works on the subject.

The studies herein recorded include: (1) The relative susceptibilities to melanose of many rutaceous plants, including citrus; (2) the

<sup>1</sup>The writers take pleasure in making acknowledgment to Ruth Colvin, E. Aline Fenner, Lulu O. Gaiser, and Harold H. Link, of the Office of Fruit Diseases; to Alfred Warren, agricultural agent, St. Lucie County, Fla.; and to H. E. Stevens, Fort Myers, Fla., formerly pathologist at the Florida experiment station, for valuable assistance rendered in laboratory and field work. Acknowledgment is made also of the very valuable cooperation by the late J. B. Magruder, Orlando, Fla., the owner of the grove in which most of the experimental spraying and dusting tests were made.

<sup>2</sup>After this bulletin was written the ascus stage of the melanose fungus was found by Frederick A. Wolf, of the Office of Fruit Diseases, and described by him as *Diaporthe citri* (Fawcett) n. n. in the Journal of Agricultural Research (25). (Italic numbers in parentheses in this bulletin refer to "Literature cited," p. 61.)



effect of temperature upon infection of potted citrus seedlings; (3) a determination by inoculation tests of the stages of development at which the leaves and fruits of commercial citrus types reach practical immunity; and (4) the results of experimental tests and of horticultural practices for melanose control.

## DESCRIPTION OF THE DISEASE<sup>3</sup>

### EFFECT ON LEAVES

Young lesions of citrus melanose occur on tender leaves first as minute, almost invisible, dark, round, sunken depressions with yellowish margins. (Pl. 1, A.) As the leaf ages the spot becomes flush with the surface and later becomes distinctly raised with a somewhat irregular margin. It is then rough and mahogany brown in color without discolored borders. (Pl. 2, B.) These lesions range from one-fourth to 1 millimeter in diameter. If the infection is severe, the leaf partially loses its natural green color and takes on a paler green or yellowish shade. The lesions occur for the most part on the upper surface of the leaf, but frequently on the lower surface as well. These melanose marks appear in various patterns. They may be widely separated single minute lesions, or they may appear in circular patterns 2 or 3 centimeters in diameter (pl. 2, C), or sometimes as solid scar tissue of various forms, and this scar tissue persists throughout the life of the leaf.

A careful histological study of citrus melanose made by Floyd (10) shows that the host tissue is killed for five or six cells in depth, and beneath this area of dead tissue specialized cells develop which cause the dead cells to be raised; hence the rough feel of melanose spots. (Pl. 3, A.)

### EFFECT ON FRUIT

Lesions on fruit (pl. 4, C, D) are similar to those on leaves. They are at first dark and sunken without discolored borders, later becoming brown, distinctly raised, rough to the touch, and usually remaining rough throughout the life of the fruit. When the infection is slight the melanose spots are usually scattered, and they may be simply brown, raised dots of about 1 millimeter or less in diameter, with or without a grayish border, or they may be very minute specks the center of which is brown with a zone of grayish, almost normal, tissue and with an outer zone of brownish melanose-scar tissue. This type is not very common, but it has been observed particularly in cases where infection occurred when the fruit passing out of its early stage was nearing immunity, under which conditions the disease usually does not develop in quantity. If the infection is severe, melanose spots are much more numerous and may appear in tear-streak patterns or as solid scar tissue, raised and rough to the touch, that may cover the greater part of the fruit. Shallow cracks of rather regular patterns develop in this dead scar tissue. This type is usually referred to as "mud-cake" melanose. (Pl. 4, E, F.) These

<sup>3</sup> Unless otherwise noted, the discussions of the disease in this bulletin relate to the orange and the grapefruit.



affected areas usually persist throughout the life of the fruit, but in some instances the brown rough tissue has a tendency to slough off, particularly in the "mud-cake" type.

#### EFFECT ON TWIGS

The infection on twigs (pl. 5, A, B) appears at first very much as it does on the leaves, and later the spots, if few in number, become much more raised than on leaves or fruit and in some instances resemble forms of die-back. If the infection is severe, "mud-cake" patterns (pl. 5, C) are likely to develop, and the twig dies. One of the distinctive features of the disease is the presence on water sprouts of two or three zones of very severe melanose infection, between which are zones comparatively or entirely free from these blemishes. These zones represent infection and noninfection periods during the growth of the water sprout.

#### DISEASES CONFUSED WITH MELANOSE

A number of blemishes are commonly confused with citrus melanose.

##### AMMONIATION

Ammoniation is considered to be a form of die-back, a common nutritional disease of citrus. These blemishes are rare on grapefruit, but on oranges they may be confused with melanose although totally different as to origin. (Pl. 6, A and B.) When the two occur on the same fruit it is difficult to distinguish between them in the field, on either mature or immature fruit, especially so when the two types occur in slight to moderate amounts. Ammoniation spots on the orange are larger, more erumpent, and usually when raised are of a lighter brown color than melanose. When these spots are examined under a hand lens, minute cracks may be found on the raised parts. These spots are usually glazed by a resinous excretion, and beneath them minute gum pockets or gum-filled areas are usually present; in very severe cases gum deposits may be found even in the central pulpy axis of the fruit.

##### STAR MELANOSE

Star melanose is a false melanose with raised dark-brown to black excrescence having irregular margin with from three to five or more points. (Pl. 7, D.) These spots range in diameter from 1 to 3 millimeters and occur more frequently on the surface of leaves than on fruit. They have been observed by the writers only on trees that previously had received applications of Bordeaux mixture or Bordeaux-oil emulsion, which suggests that they are probably due to spray injury.

##### BLACK MELANOSE

Black melanose is a spotting of citrus leaves which, although not similar to citrus melanose in appearance, causes considerable confusion because of the similarity of names. Black melanose appears at first as a yellowish green discoloration on orange and grapefruit leaves. These spots soon become distinctly raised on both surfaces, owing to an accumulation of resinous material in the affected area,



and gradually become dark colored, sometimes almost black. They vary considerably in size, usually ranging from  $1\frac{1}{2}$  to 4 millimeters in diameter. Generally the margin on the upper surface is regular in outline, but on the under surface it may be either regular or irregular (pl. 7, C). The cause of this disorder has not been established. It does not seem to be infectious, as no fungus or other organism has been found associated consistently with the spots.

Another spotting on leaves and fruit that is similar to melanose has been observed in Alabama and Texas on trees lacking vigor. It so closely resembles melanose that the two may easily be confused. In this type of injury the margin of the affected tissue is more regular, the excrescences much higher, and the color darker than in citrus melanose.

#### RUST-MITE TEAR STAIN

Rust-mite tear stain is frequently confused with melanose tear streak. Both occur on oranges and grapefruit and both are mahogany brown in color. The melanose tear streak is rough to the touch and raised, while the rust-mite tear stain is smooth and flush with the surface of the unaffected parts.

#### RUST-MITE RUSSET

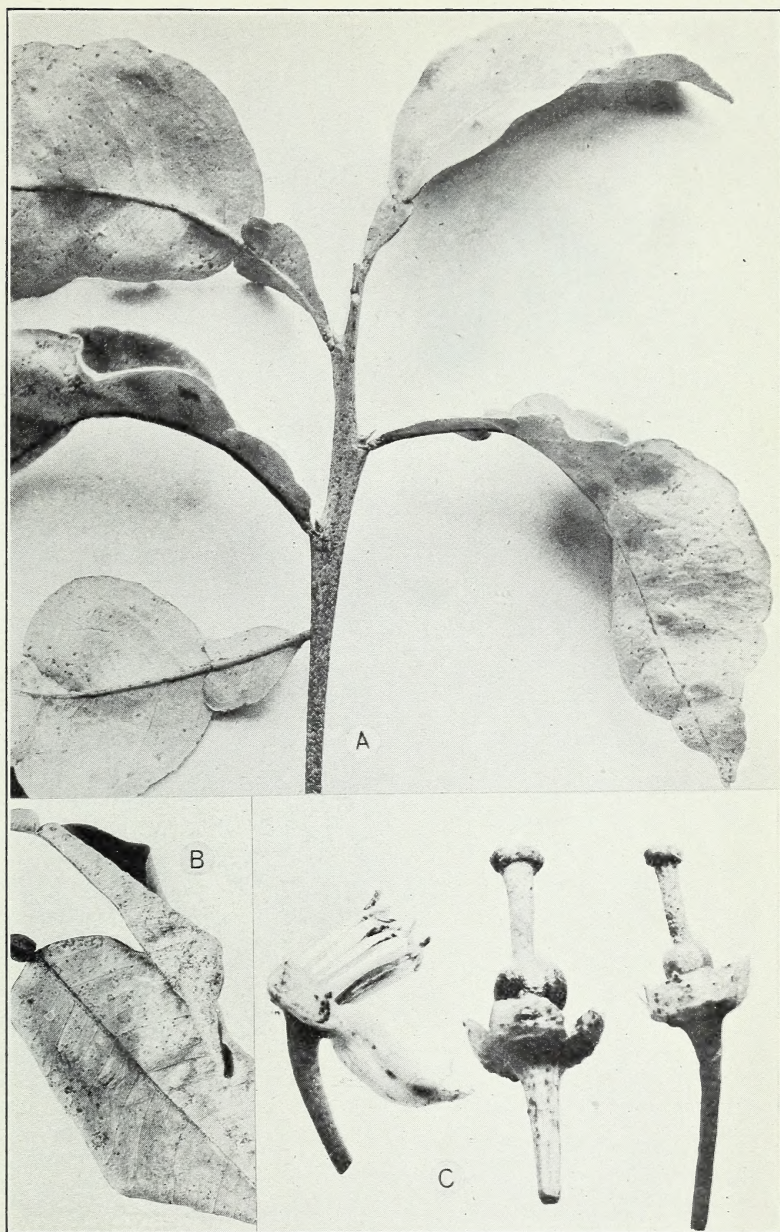
Rust-mite russet frequently occurs on oranges and grapefruit as a general discoloration or as distinct zones or bands that are usually quite smooth to the touch, but occasionally they may be slightly rough, faintly resembling "mud-cake" melanose. When the rust-mite russet is severe it is impossible to detect with accuracy a slight to moderate occurrence of melanose lesions in the same general area.

#### HISTORICAL REVIEW

The first published record of citrus melanose was made by Swingle and Webber (21) in 1896. They first observed it in November, 1892, near Citra, Fla. The hosts recorded in order of susceptibility were grapefruit, sweet orange, sour orange, lemon, mandarin, and Satsuma. The disease when first observed seemed to be of very recent origin or introduction, as evidenced by its limited distribution, but it spread rapidly during the next few years. They occasionally found microscopic evidence of fungal hyphæ in the affected parts, but were unable to obtain the fungus in culture. They considered the disease to be due to a "vegetable parasite." Spraying experiments conducted in 1894 at Stanton, Fla., showed that the disease was controlled by two applications of Bordeaux mixture or of ammoniacal solution of copper carbonate, the first made about two weeks after the flowers had fallen and the second about a month later.

Floyd (10) concludes, after studying the disease from a physiological and histological standpoint, that it is caused by some unrecognized organism, and states "it is probable that the dead wood is a source of infection." He observed that fruit so located that water from deadwood drops upon it often has a tear-stain arrangement of the disease marking. He also noted that where melanose had been severe, pruning proved to be helpful in reducing the disease.

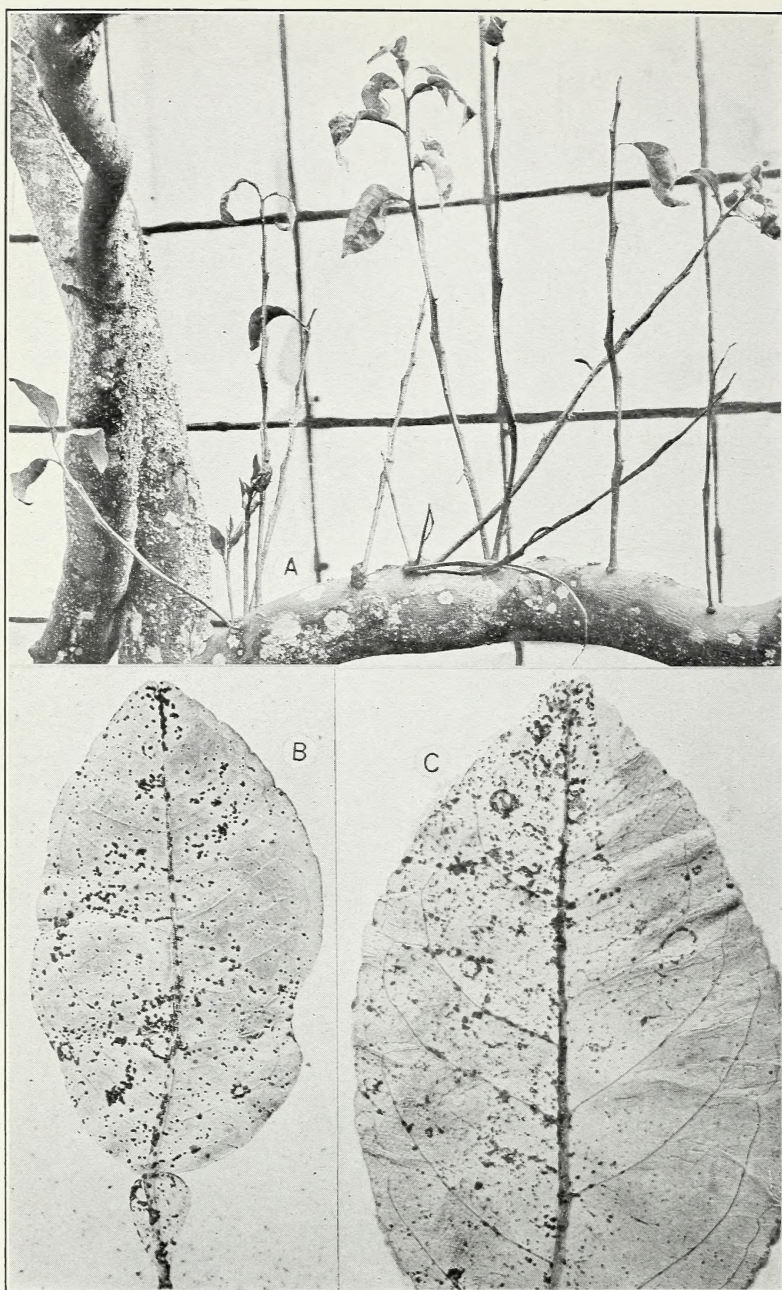




EXAMPLES OF MELANOSE INFECTION.—I

- A, Early, slightly sunken stage of natural melanose infection on young grapefruit leaves and twig  
 B, Orange leaves curled from melanose infections. These leaves will fall prematurely  
 C, Melanose lesions on petal, calyx, and fruit stem of grapefruit

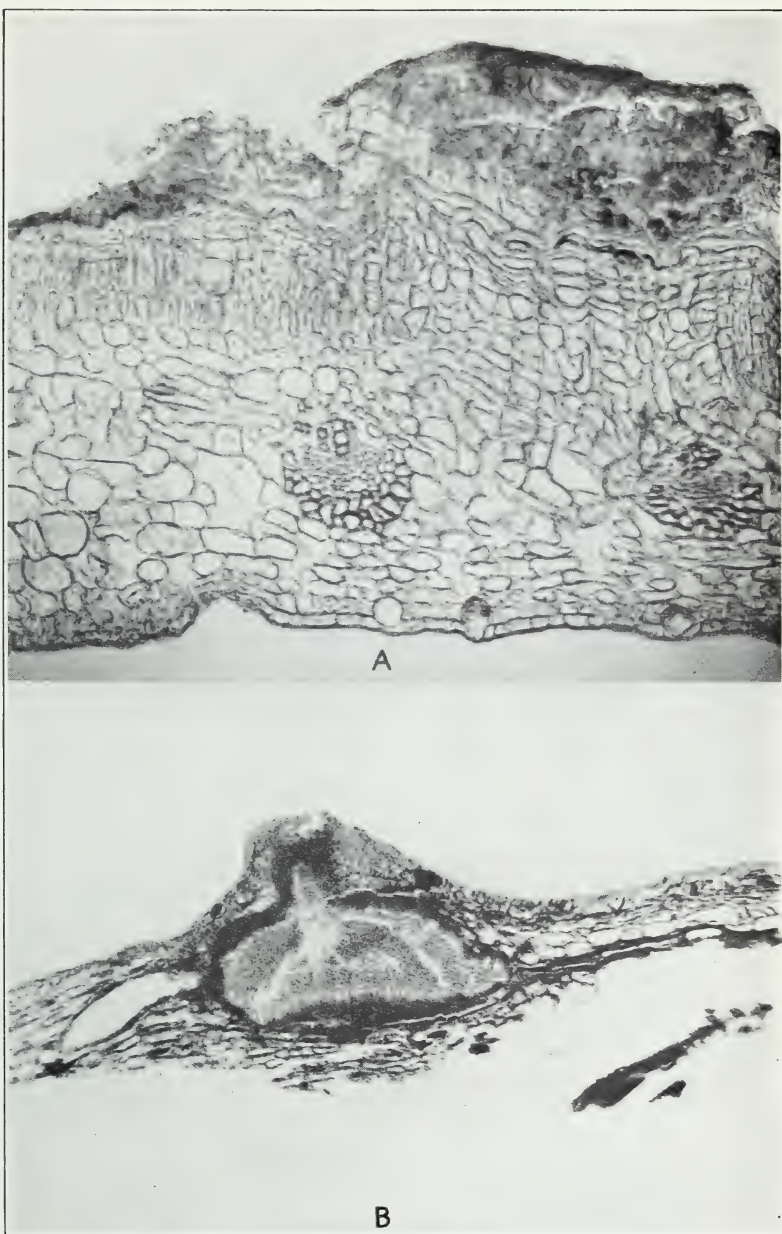




#### EXAMPLES OF MELANOSE INFECTION.—II

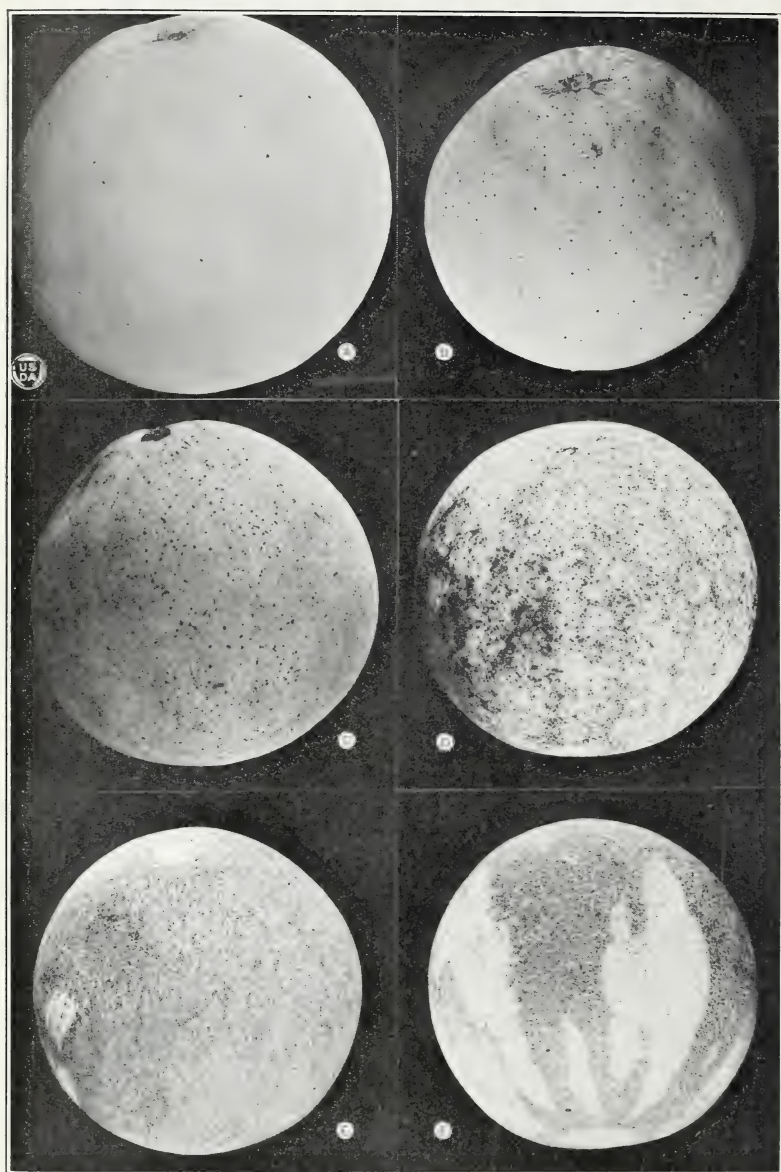
- A, Defoliation of grapefruit water sprouts caused by severe melanose infection. Such twigs usually die as a result of the disease and later become foci of infection  
 B, Grapefruit leaf, showing old melanose spots  
 C, Natural melanose infection of bergamot orange leaf, showing circular arrangement of lesions





**MELANOSE CROSS SECTIONS**

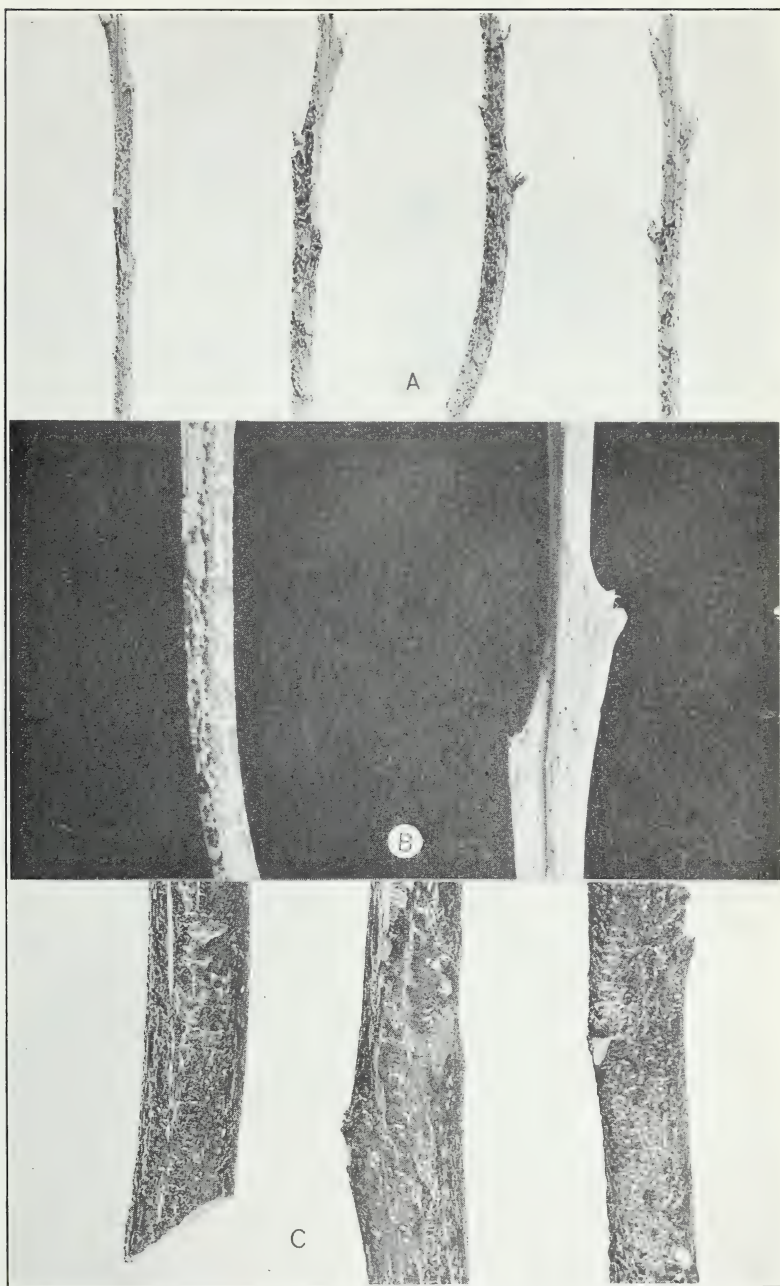
- A, Cross section through an old melanose spot on a leaf ( $\times$  about 200). Note the raised portion of the tissue heavily infiltrated with dark gum  
B, Cross section through a pycnidium of the melanose fungus ( $\times$  about 100)



VARIOUS DEGREES OF MELANOSE INFECTION ON GRAPEFRUIT

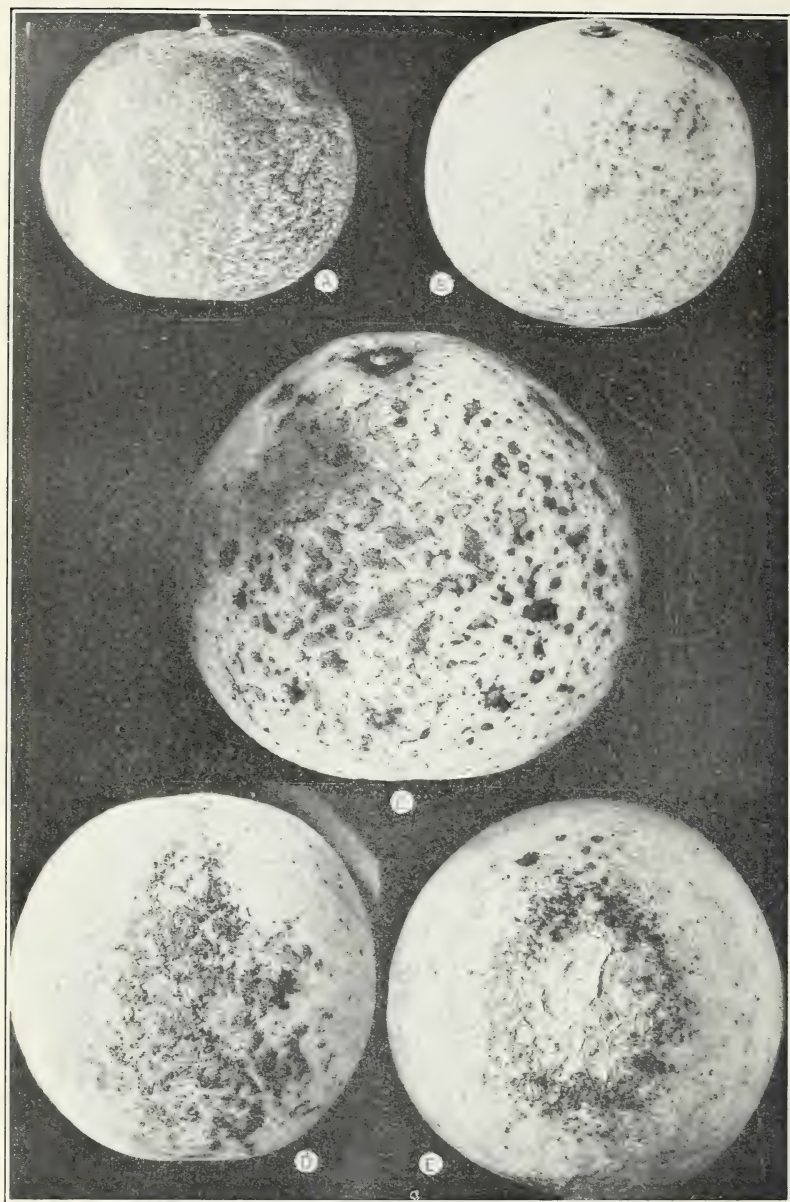
A, Very slight; B, slight; C, medium; D, severe; E, very severe; F, tear-streak pattern of melanose, much of which is of the "mud-cake" type





**MELANOSE ON ORANGE AND GRAPEFRUIT TWIGS**

- A, Severe melanose infection on very young grapefruit twigs. Such lesions may easily be confused with "ammoniation"  
B, Typical melanose lesions on orange twigs  
C, "Mud-cake" effect of melanose on orange twigs



#### TYPES OF INJURY ON ORANGES AND GRAPEFRUIT

A and B, Oranges affected with "ammoniation." This blemish is easily confused with melanose.

C, Grapefruit showing Bordeaux-oil injury. This is a rather common type of spray injury.

D, An unusual type of Bordeaux-oil injury on orange. It has been observed on experimentally sprayed plots only, and there but rarely.

E, Sulphur sunburn on grapefruit. This type of injury occurs commonly during the summer months following applications of sulphur sprays. Applications of sulphur within about 10 days before or after an application of oil emulsion is almost certain to be followed by this type of burn.



Floyd and Stevens (11) state that melanose is caused by *Phomopsis citri* Fawcett, which sporulates in deadwood, and since the fungus is active nearly the entire year, melanose may occur at any time when new growth is present. Inoculation experiments with spores from pure cultures of *P. citri* produced the disease, but no spotting resulted from using the mycelium or filtrate from twig washings and cultures. They were unable to recover the causal organism from melanose markings.

Fawcett (5, 6) had previously shown that *P. citri* causes a stem-end decay of citrus fruits.

Stevens (18) gives the results of pruning experiments in 1913 and 1914 for melanose control in a grove that had suffered severely from melanose, showing that very careful pruning reduces the disease decidedly and that ordinary commercial pruning gives a moderately good control.

Fawcett (7) calls attention to the distribution of melanose in Florida. He states that this disease is confined roughly between  $27\frac{1}{2}^{\circ}$  and  $29\frac{1}{2}^{\circ}$  north latitude. North and south of these limits it becomes progressively less prevalent. He suggests that climatic conditions greatly influence the distribution of melanose.

Stevens (19) obtained further positive inoculations from pure cultures of *P. citri*, but was unable to reisolate the causal organism from lesions or to detect it in microscopic examination. He reports further on four years of successful pruning experiments for melanose control.

From the development of this disease on plots of grapefruit sprayed in 1914 and 1915 for citrus scab control, Grossenbacher (12) concludes that one application of Bordeaux-oil emulsion made in late April or early May usually gives good protection against melanose, but occasionally, when April is rainy, two applications should be made, the first about the middle of April and the second about the middle of May.

Burger, DeBusk, and Briggs (2) failed to produce melanose infection on young oranges and grapefruit picked on May 20 and placed in a moist chamber in the laboratory. From these laboratory tests, together with general observations, they conclude that fruit becomes immune about June 1. From spraying tests conducted during 1921 and 1922 they conclude that fruit sprayed from 10 to 20 days after the bloom has dropped will be protected from this disease.

Winston and Bowman (24) give the results of spraying experiments conducted over several years, beginning in 1920, and particularly the results obtained in commercial grove spraying in 1922. They conclude that in normal years a single application of standard Bordeaux-oil emulsion spray completed by May 5 will give good commercial control of the disease, but in years especially favorable for the disease two applications may be necessary. Under such conditions they suggest that the first application be made between April 1 and 10 and the second about one month later.

Burger (1) is the first to report the occurrence of what he considered *P. citri* in California, where it was found to be producing stem-end rot of citrus fruits.

Fawcett (9) reports finding pycnidia of *Phomopsis* in California and suggestions of melanose on citrus leaves. Fruit picked from branches containing dead stems developed typical stem-end decay. This California *Phomopsis* seemed sufficiently distinct from *P. citri* to be described as a new species, to which he gave the name *Phomopsis californica* n. sp.

Dozier (4) states that melanose is of rather rare occurrence on the Satsuma in the Gulf coast region, but during the summer following the severe freeze of 1924 this disease developed on Satsuma leaves in Alabama.

Fawcett (8) shows that *P. citri* makes its most rapid growth on culture media at about  $27\frac{1}{2}^{\circ}$  C., but makes a good growth at temperatures as low as  $18.5^{\circ}$  and as high as  $30^{\circ}$ . Above  $32^{\circ}$  no growth was observed, and at  $7.5^{\circ}$  only slight growth took place over the period of observation.

Cobb (3) mentions a disease in Australia resembling melanose in many respects. He found in the affected spots a distinct fungus which he illustrated, but he failed to name or describe it. He believed this fungus to be the cause of the disease. Later, McAlpine (15) described this organism as *Cladosporium brunneo-atrum* n. sp. McAlpine states that the Australian disease attacks the sweet orange only, whereas the Florida disease is known to attack many species of citrus. Pathological differences between the two diseases are recorded. Owen (16) reports the successful control of the Australian melanose with applications of Bordeaux mixture.

Trabut (23) reports the occurrence of a melanose disease on mandarins and in these spots found a fungus which he considers the cause of the disease. To this fungus he gives the nomen nudum *Septoria glaucens*, but he fails to state its habitat.

Lee (14), while conducting spraying experiments in Japan on citrus, found a blemish identical in appearance with citrus melanose that developed to a considerable extent on trees sprayed with Bordeaux mixture and Burgundy mixture, whereas few, if any, blemishes developed on those sprayed with formalin or with lime-sulphur solution, or even on the unsprayed checks. From the writer's experience in Florida this blemish would seem to be spray injury.

Horne (13) mentions finding typical melanose spots on grapefruit from the Isle of Pines, West Indies, and later when the fruit went down with stem-end decay he isolated *Phomopsis* from the affected tissue. Laboratory studies showed this organism to be distinct from *Phomopsis citri* Fawcett. He describes this new fungus and suggests for it the name *Phomopsis caribaea* n. sp.

It thus seems that there are in the literature at least four distinct markings, each of which is called melanose, and which appear to be caused by different agents; namely, the Florida disease, caused by *P. citri* Fawcett; Traub's disease, presumably caused by the fungus *Septoria glaucens* Traub, nomen nudum; the Australian disease, attributed to *Cladosporium brunneo-atrum* McAlpine; and the Japanese blemish, which is probably a type of spray burn. On the basis of priority it would seem that the Florida disease should be entitled to the name melanose. There is also the possibility that two other species of *Phomopsis*, *P. californica* and *P. caribaea*, produce melanose blemishes.



Considering the importance of citrus melanose, very little work seems to have been done on the subject either in the laboratory or in the grove, and that which has been published has until recently had but little effect upon the practical control of the disease. Prior to 1921 citrus melanose was generally considered to be one of the many diseases that could not be controlled economically with fungicides, because the harmful effects in the form of excessive increase in scale insects outweighed all beneficial effects resulting from a reduction of melanose. This increase in scale insects is due to the effect of the fungicide upon entomogenous fungi that attack these insects, thereby upsetting the natural biological scale control. With the introduction of Bordeaux-oil emulsion this objectionable feature was largely overcome. Commercial pruning has usually failed to give satisfactory control.

### SPECIES AND VARIETIES ATTACKED

Citrus melanose occurs on a wide range of plants of the family Rutaceae, to which citrus belongs, and it seems that all commercial varieties of citrus grown under Florida conditions are attacked by the disease. Among the citrus varieties grown commercially, the kumquats<sup>4</sup> (*Fortunella* spp.) are perhaps the most susceptible; they are extremely so. The lemons (*Citrus limonia* Osbeck), sour oranges (*C. aurantium* L.), limes (*C. aurantifolia* Swingle), and grapefruit (*C. grandis* Osbeck) are very susceptible, whereas the round (sweet) oranges (*C. sinensis* Osbeck) and the kid-glove oranges (*C. nobilis* Lour.), although distinctly less susceptible than the grapefruits, are attacked severely whenever conditions are especially favorable for infection. Observations over a period of eight years and inoculations made during six years fail to reveal any appreciable difference in the degree of susceptibility possessed by the commercial varieties within the same species. Susceptibility seems to be possessed to about the same degree by the fruit, the leaves, and the twigs.

In addition to the commercial varieties of citrus found throughout the citrus belt, studies by means of inoculation tests have been conducted on the susceptibility to melanose attack of a rather wide range of rutaceous plants, including a number of citrus hybrids. These include representatives of 21 genera and 34 species. In some instances only one or two plants of a given species have been available; hence the susceptibility of these is recorded tentatively. More complete data resulting from inoculation tests will be found in that portion of the bulletin which deals with inoculation experiments. Table 1 gives the approximate susceptibility to melanose of a wide range of rutaceous plants under Florida conditions as indicated by extensive inoculation experiments and general observations.

<sup>4</sup>In so far as possible, the botanical classification of citrus species used in this bulletin follows that of W. T. Swingle (22).

TABLE 1.—Range of infection on various rutaceous plants by the melanose fungus *Phomopsis citri* Fawcett, as indicated by artificial inoculations and general observations

Botanical name	Common or variety name	Range of infection
<b>Family Rutaceae:</b>		
<b>Subfamily Citratae—</b>		
<i>Glycosmis pentaphylla</i> DC.....	.....	Severe.
<i>Claucena lansium</i> Sk.....	.....	Susceptible.
<i>Chalcas (Murræa) exotica</i> Mills.....	.....	No infection observed.
<b>Tribe Citreae—</b>		
<b>Subtribe Aeglinæ—</b>		
<i>Feronia limonia</i> Sw.....	.....	Do.
<i>Feroniella lucida</i> Sw.....	.....	Do.
<i>Aeglopsis chevalieri</i> Sw.....	.....	Slight.
<i>Aegle marmelos</i> Cor.....	.....	No infection observed.
<i>Chaetosperrum glaucinosa</i> Sw.....	.....	Medium.
<i>Balsamocitrus gabonensis</i> Sw.....	.....	No infection observed.
<b>Subtribe Lavanginæ—</b>		
<i>Hesperethusa crenulata</i> Roem.....	.....	Do.
<i>Triphasia trifolia</i> P. Wilson.....	.....	Very slight.
<i>Paramigyna monophylla</i> Wight.....	.....	No infection observed.
<i>Severinia buxifolia</i> Ten.....	.....	Very slight.
<b>Subtribe Citrinæ—</b>		
<i>Citropsis schweinfurthii</i> S. and K.....	.....	No infection observed.
<i>Poncirus (Citrus) trifoliata</i> Raf.....	.....	Slight.
<i>Atalantia citroides</i> Pierre.....	.....	No infection observed.
<i>Fortunella hindsii</i> Sw.....	.....	Susceptible.
<i>Fortunella margarita</i> Sw.....	Nagami kumquat (oval).....	Very severe.
<i>Fortunella japonica</i> Sw.....	Marumi kumquat (round).....	Do.
<i>Fortunella crassifolia</i> Sw.....	Meiwa kumquat.....	Do.
<i>Citrus ichangensis</i> Sw.....	.....	Susceptible.
<i>Citrus medica</i> L.....	Corsican citron.....	Slight to medium.
Do.....	Ettog citron.....	Do.
<i>Citrus limonia</i> Osbeck.....	Meyer lemon.....	Severe to very severe.
Do.....	Kennedy lemon.....	Do.
Do.....	Lamb lemon.....	Do.
Do.....	Rough lemon.....	Do.
Do.....	Sweet lemon.....	Do.
Do.....	Villafranca lemon.....	Do.
<i>Citrus janos</i> Tan.....	Kansu orange.....	Do.
<i>Citrus aurantifolia</i> Sw.....	Kansai lime.....	Do.
Do.....	Mexican lime.....	Do.
Do.....	Persian lime.....	Do.
Do.....	Sour Rangpur lime.....	Do.
Do.....	Tahiti lime.....	Do.
Do.....	Thornless lime.....	Do.
Do.....	Woglum lime.....	Do.
<b>GRAPEFRUIT GROUP</b>		
<i>Citrus grandis</i> Osbeck.....	Conner grapefruit.....	Severe to very severe.
Do.....	Davis grapefruit.....	Do.
Do.....	Duncan grapefruit.....	Do.
Do.....	Foster grapefruit.....	Do.
Do.....	Gold Medal grapefruit.....	Do.
Do.....	Hall (Silver Cluster) grapefruit.....	Do.
Do.....	Leonardi grapefruit.....	Do.
Do.....	Marsh grapefruit.....	Do.
Do.....	McCarty grapefruit.....	Do.
Do.....	Pink Marsh grapefruit.....	Do.
Do.....	Pernambuco grapefruit.....	Do.
Do.....	Royal grapefruit.....	About the same as orange.
Do.....	Triumph grapefruit.....	Do.
Do.....	Chinese pummelo.....	Severe to very severe.
Do.....	Pink pummelo.....	Do.
Do.....	Sour pummelo.....	Do.
Do.....	Common shaddock.....	Do.
Do.....	Cuban shaddock.....	Do.
<b>SOUR-ORANGE GROUP</b>		
<i>Citrus aurantium</i> L.....	Bergamot orange.....	Severe to very severe.
Do.....	Bittersweet orange.....	Do.
Do.....	Myrtle-leaf orange.....	Do.
Do.....	Willow-leaf bittersweet orange.....	Do.
Do.....	Otaheite orange.....	Do.
Do.....	Sour orange.....	Do.



TABLE 1.—Range of infection on various rutaceous plants by the melanose fungus *Phomopsis citri* Fawcett, as indicated by artificial inoculations and general observations—Continued

Botanical name	Common or variety name	Range of infection
<b>ROUND OR SWEET ORANGE GROUP</b>		
<i>Citrus sinensis</i> Osbeck	Chamoudi orange	Severe to very severe, but distinctly less so than grapefruit.
Do.	Florida seedling orange	Do.
Do.	Enterprise orange	Do.
Do.	Homosassa orange	Do.
Do.	Jaffa orange	Do.
Do.	Lamb orange	Do.
Do.	Lue orange	Do.
Do.	Maltese orange	Do.
Do.	Mediterranean Sweet orange	Do.
Do.	Norris early orange	Do.
Do.	Parson Brown orange	Do.
Do.	Pineapple orange	Do.
Do.	Ruby orange	Do.
Do.	Surprise orange	Do.
Do.	Valencia orange	Do.
Do.	Washington Navel orange	Do.
<b>KID-GLOVE ORANGE GROUP</b>		
<i>Citrus nobilis</i> Lour	King orange	Slightly less than sweet orange.
<i>Citrus nobilis deliciosa</i> Sw	Clementine orange	Do.
Do.	Cleopatra mandarin	Do.
Do.	Dancy tangerine	Do.
Do.	Oneco tangerine	Do.
Do.	Mandarin	Do.
<i>Citrus nobilis unshin</i> Sw	Satsuma	Do.
<i>Citrus mitis</i> Blanco	Calamondin	Do.
<i>Citrus (Papeda) hystrix</i> DC	Pointed-leaf Papeda	Susceptible.
Do.	Round-leaf Papeda	Do.
<i>Citrus webberi</i> Webster	Round-leaf Papeda	Do.
<i>Microcitrus australasica</i> Sw		No infection observed.

The designation of degree of infection in Table 1 follows as closely as possible the scale used elsewhere in this bulletin for recording results of the inoculation and spraying experiments.

From this table it is seen that melanose develops on a very wide range of plants in family Rutaceae, subfamily Citratae. Of the 34 species under observation, the disease developed on all except 10, and these apparently immune members are scattered quite well throughout the entire range. *Toddalia lanceolata* Lam., the only form under study in subfamily Toddalioidae, is also apparently immune.

*Triphasia trifoliata* and *Severinia buxifolia* are attacked very slightly, and the citrons, kid-glove oranges, round oranges, limes, lemons, sour oranges, grapefruit, and kumquats are affected in about the order named. Among the grapefruits the Royal and Triumph varieties, as a rule, are less severely blemished than the average commercial grapefruit. These two varieties are supposed to be hybrids between grapefruit and orange.

According to the observations of the writers, the greater susceptibility of grapefruit to infection is shown by the fact that it is much easier to find melanose scars on leaves and small green grapefruits than on orange fruits and leaves of the same age, and that the degree of injury is almost always more severe on grapefruits. Furthermore, the causal organism produces pycnidia in nature more

freely on the dead grapefruit twigs than on the orange. This latter condition would indicate that the grapefruit is subject to a heavier spore barrage than the orange, and the former would then have a greater mathematical chance to become infected. This alone might account for an appreciable amount of infection in excess of that occurring on the orange, but the results of inoculation experiments reported in Tables 3 and 4 indicate that the grapefruit is inherently more susceptible than the orange.

Of the 21 hybrids under observation, all were found to be susceptible to melanose. The disease has been observed on them in approximately the following order:

#### PRESENT

Citrardia (trifoliolate orange and sour orange).  
 Limelo (Rangpur lime and sour pummelo).  
 Limelo (Mexican lime and sour grapefruit).

#### SLIGHT TO MEDIUM

Rusk citrange (trifoliolate orange and sweet orange).  
 Citremon (Lisbon lemon and trifoliolate orange).  
 Natsumikan (probably pummelo and a kid-glove orange).  
 Siamelo (King orange and grapefruit).  
 Tangelos, Sampson and Thornton (tangerine and grapefruit).  
 Tangor (tangerine and orange).

#### SEVERE TO VERY SEVERE

Thomasville citrangequat (Nagami kumquat and Willits citrange).  
 Faustrime (Mexican lime and Australian finger lime).  
 Faustrimedon (Australian finger lime and calamondin).  
 Lemelo or lempum (Chinese pummelo and Myers lemon).  
 Lemon hybrid (probably lemon and citron).  
 Lemonime (Mexican lime and Genoa lemon).  
 Lemonquat (Genoa lemon and Nagami kumquat).  
 Limequat (Mexican lime and Marumi kumquat).  
 Microcitrus hybrid (probably Australian finger lime and Fooji lime).  
 Orangelo (grapefruit and orange).  
 Tangelolo (grapefruit and Sampson tangelo).

The kumquat hybrids are very readily blemished, more so than most grapefruits and at least as severely as the kumquats, but the tangelos as a class seem to be even less susceptible than the tangerine.

### GEOGRAPHIC DISTRIBUTION

#### REPORTED OCCURRENCES

Since there are several diseases that can easily be confused with citrus melanose, it is probable that some of the reports of the occurrence of this disease in foreign countries, and even in America, may be erroneous. It is evident that the melanose of mandarins as reported by Trabut and the false melanose of sweet oranges in Australia as described by McAlpine are distinct from the American disease, and probably Lee in Japan mistook spray injury for melanose.

Citrus melanose, therefore, seems to be confined to the Americas, including the West Indies. It has been reported from Brazil, the British West Indies, Porto Rico, Isle of Pines, Cuba, and Mexico, and in the United States in the States of Florida, Alabama, Mississippi, Louisiana, Texas, and California.



In Brazil, the West Indies, and California melanose is reported to occur in negligible quantities, whereas in Texas, Louisiana, Mississippi, and Alabama the disease is said to be sporadic in its behavior; sometimes it occurs in serious proportions, but generally it is slight.

#### LOCAL DISTRIBUTION IN FLORIDA

Although citrus melanose occurs in varying proportions throughout the citrus regions of the Gulf Coast States, the disease occurs at its worst in central Florida, where it is regularly a serious disease, varying in intensity from locality to locality, and somewhat from year to year. Melanose is generally distributed throughout the citrus belt of Florida, where it is a serious disease in most of the old citrus districts and is becoming gradually more serious where it has heretofore occurred in minor amounts. Wherever the groves are relatively young the disease is not usually serious, but it is becoming progressively more abundant with the increasing age of the plantings, and special measures are being taken to control it. In flatwoods, hammocks, or other low and damp locations citrus melanose occurs in abundance, and many of the older groves of Florida are set in such locations. It has not been found to be severe on wild citrus growing in densely shaded hammocks, where the citrus trees grow very slowly but have a healthy color of foliage. In many instances the disease is more pronounced in inland sections than close to the seashore.

Melanose has not yet become a chronically serious disease in the extreme southern citrus districts of the State, although it caused an enormous damage in that general territory following the freeze of 1917. This is strong evidence that if conditions are favorable for outbreaks citrus melanose should be expected to become a serious menace, for a limited time at least, in any fruit district in southern Florida. In the northern part of the citrus belt of Florida, especially where there is occasional damage by freezes, it becomes especially severe following cold winters.

Figure 1 shows the principal regions of melanose infection, which coincide roughly with the districts where the round orange and the grapefruit are grown commercially. In the northern and western parts of the State extensive developments in Satsuma planting are taking place, but the industry has not become stable there, and for that reason they are not shown on this map, although melanose occurs there to some extent.

It will be seen from this map that melanose is an important disease throughout the entire round-orange and grapefruit belt. On the high, dry lands through the central part of the peninsula where most of the groves are relatively young and along the lower east and west coast the disease has not yet become generally serious.

#### ECONOMIC IMPORTANCE

In general importance to the citrus industry of the world, citrus melanose appears to be of minor significance, but it is a major factor to the citrus industry of Florida. In the districts where the disease occurs in quantity, citrus melanose, together with the *Phomopsis*

type of stem-end rot, either actually renders unfit for human consumption or impairs the market value of more citrus fruit than all other active parasitic diseases combined. The damage resulting from *Phomopsis* infection may be divided into three general classes:

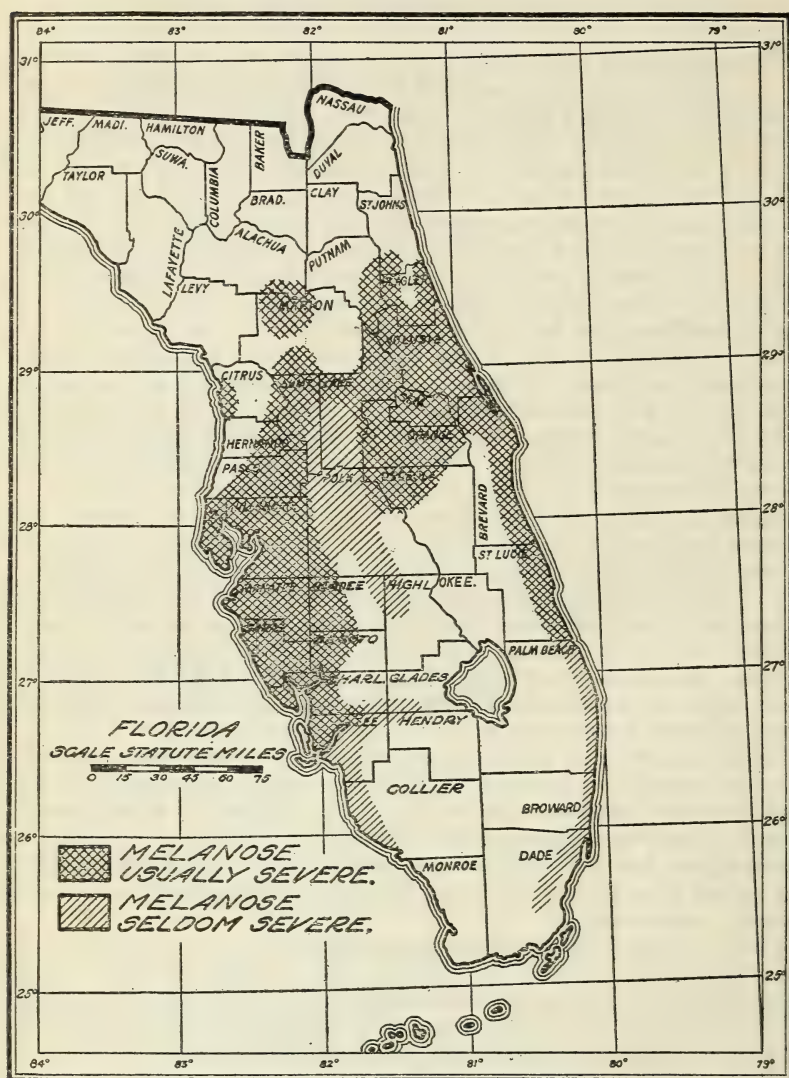


FIG. 1.—Map of Florida, showing by shading the round-orange and grapefruit belt and the principal regions of melanose infection. Citrus plantings are found at many scattered points outside the shaded areas

- (1) Injury to the tree in the form of leaf and twig blemishes which in severe cases result in twig defoliation and death of tender twig growth.
- (2) Melanose scars on fruit that reduce or may even destroy the sale value of a large part of the crop.
- (3) Stem-end decay of fruit, that may develop in the grove, in transit, on the market, or after the fruit reaches the consumer.



## TREE INJURY

The injury to trees resulting from a slight leaf infection is usually of no special consequence, but severe infection frequently causes a crinkling of the leaves (pl. 1, A and B) which impairs their function, and in the more severe outbreaks defoliation and killing back of twigs (pl. 2, A) result. This severe injury is likely to develop in the spring and summer following frosts or freezes that defoliate and kill twigs. When this blighting occurs in quantity the prospect for a heavy crop the following year is unfavorable. Obviously the financial losses resulting from such a form of injury are difficult to estimate.

## FRUIT BLEMISH

Melanose blemishes on fruit (pl. 4) are responsible for a tangible financial loss, as is shown by the reduction in the market value of fruit bearing melanose markings. A fruit with a moderate amount of melanose is usually packed in the second or third grade, but a severely blemished fruit is a cull, and therefore has no market value. Severe melanose infections may even cause the dropping of fruits  $1\frac{1}{2}$  inches or less in diameter, but this melanose-induced drop occurs in quantity rather infrequently.

## FRUIT DECAY

Stem-end rot, caused by the melanose fungus, is responsible for fully 40 per cent of the decay of Florida oranges and grapefruit in transit, on the market, or in the consumer's home. This phase is not to be discussed in this bulletin, but is mentioned simply to call attention to the potential importance of melanose and its usual sequel. There is also an important stem-end rot of Florida citrus fruits caused by *Diplodia natalensis* Pole Evans.

In 1922 the State of Florida produced more than 25,000,000 bushels of oranges and grapefruit. Fully 15,000,000 bushels of those bore melanose blemishes to a greater or less degree, and at least 1,000,000 bushels were scarred by this disease in sufficient proportions to cause them to be packed in the third grade or lower, or even disposed of as culls by those who put up a carefully graded pack.

The importance of this disease in nurseries and young groves is usually not great, but following damaging frosts or other conditions which tend to increase the amount of deadwood, melanose may do considerable damage to new shoots. Therefore it is plainly evident that the melanose fungus is not only potentially capable of causing enormous losses but actually does cause these heavy losses. With the citrus crop of Florida increasing rapidly, it is only reasonable to assume that the losses attributable to the fungus *Phomopsis citri*, namely, melanose and stem-end rot, will likewise increase unless special measures are taken to prevent them.

Although this blemish reduces the sale value of the fruit, the taste is not noticeably altered, but fruit bearing melanose scars is more likely to decay ultimately from stem-end rot than fruit free from such blemishes.

## THE CAUSAL ORGANISM

The inability to recover *Phomopsis citri* Fawcett from either naturally or artificially induced melanose markings makes it impossible to follow strictly Koch's postulates for proof of pathogenicity; but, on the other hand, cumulative evidence, including extensive inoculation experiments, goes to prove beyond a reasonable doubt that citrus melanose as it occurs in Florida is caused by this fungus, which also causes a stem-end decay of a wide range of citrus fruits. In the latter case the four postulates of Koch are easily followed.

The ascogenous form of this fungus has not been found.<sup>5</sup> *P. citri* frequently produces two types of "spores," the one a short, broad, functional conidium, the other a long, slender, hooked body of undetermined origin and function, referred to in the literature variously as scolecospore, stylospore, beta spore, and paraphysis. These slender bodies appear to be sterile, as indicated by their failure to germinate in common culture media such as corn-meal agar and potato-dextrose agar. The ratio of the two types of "spores" varies greatly even from one pycnidium to another developing from single spore cultures. In some pycnidia these scolecospores appear to be absent, whereas in others they occur in numbers about equal to or even many times more numerous than the functional spores. In most instances they occur in proportions varying from 1 to 20 to 1 to 1 or less, with an average of about 1 to 4 or 1 to 5.

In nature, pycnidia without scolecospores, presumably of the melanose fungus, are found frequently, but when present they occur in as widely varying proportions as is the case in pure cultures.

Measurements of spores from pure cultures of *P. citri* grown on corn-meal agar and on sterile grapefruit stems were made for comparison with those produced in nature on dead citrus twigs, as well as those grown in the rind of green grapefruits. More than 100 spores were measured from each source, and little or no difference in size was noted regardless of whether the spores were grown in pure culture or produced in nature. The spores ranged from 6 to 8 microns in length, averaging about 7 microns, and from 2 to 3 microns in width, averaging about 2.5 microns.

The scolecospores vary in length from 22 to 28 microns and in width from 0.7 to 1.4 microns.

Pycnidia of the same fungus produced in the bark of grapefruit stems were also measured (pl. 3, B). These bodies are very irregular in outline, and therefore the measurements were variable. However, the outside dimensions of pycnidia of *P. citri* are approximately 250 to 600 microns by 125 to 325 microns.

Pycnidia of the causal organism are difficult to detect in nature without the aid of a hand lens. They occur sometimes in the dead bark of the largest limbs or even trunks, but they are found most abundantly on the small twigs and fruit stems of the preceding year's crop, as well as on the pedicels of young fruit which dropped in late spring or early summer. These fruiting bodies (pl. 8, B) are found embedded in the outer bark of dead parts with only the ostiole visible, which, when the fungus is fruiting, appears as a minute pimple with a light or cream-colored head or with a stringy tendril. If the

<sup>5</sup> See footnote 2 (p. 1).



pycnidia are young but not liberating spores, the visible portions are usually dark or almost black; if the pycnidia are very young, a small pimple in the bark is all that can be seen.

If there is a point where the organism sporulates more abundantly than another, it is somewhat below the leaf scars on twigs, especially in patches of bark that have turned white. Spore production is more abundant in tissue that has recently died than in those parts that have been dead for a year or more. Old weathered pycnidia usually show no signs of escaping spores. Many of these pycnidia, together with the surrounding bark, either partially weather away or drop out in a relatively short time, leaving empty cavities in the bark. In general it is less difficult to find inactive pycnidia than sporulating ones.

This fungus produces spores in immature citrus fruits artificially inoculated, but spore production has not been observed in mature citrus fruits under natural conditions. During the last several years careful examinations have been made of the remains of mature oranges and grapefruit under bearing trees and on cull piles in all stages of active decomposition, as well as of the mummified hulls, without finding indications of pycnidia of the causal organism.

The melanose fungus in nature sporulates principally in dead twigs and fruit stems. Deadwood in citrus trees may be the result of a wide range of conditions. Among the principal ones are infestations of scale insects; shading effects in the interior of the tree; the slow, wasting trunk and root diseases which frequently result in the death of twigs, limbs, or even trees; frosts or freezes, storms and droughts, and other conditions which tend to lower the vitality.

Under Florida conditions, and perhaps in most subtropical regions, scale insects are usually responsible for the death of more twigs than are all other agencies combined. Whenever the infestation is heavy, even for a short time, large proportions of the bearing wood are killed, and these insects, even when present in almost unnoticeable numbers, are responsible for the death of many twigs and small branches.

Aside from the agencies causing the accumulation of deadwood, the length of time the parts have been dead with respect to the time when melanose outbreaks occur is of considerable importance. Parts that have died within two or three months are much more likely to harbor the causal organism than parts that have been dead many months. Those parts killed as late as September or October seldom are a source of infection that year, and normally do not act as especially fertile sources of infection the following year, but those killed in winter or spring almost invariably are excellent breeding places for the melanose fungus. Normally very little wood is killed during the summer, but that which is killed then is a source of fall infection.

Fruiting pycnidia may be found at any time of the year, but usually they are not abundant in midwinter. Ordinarily as spring comes on innumerable pycnidia begin to develop, and around the middle or latter part of April they are found in abundance, many of them filled with spores, whereas others seem to be in the very first stages of development. Well after May rains set in, these mature fruiting bodies are not so easily found unless wet, but inactive

ones become more plainly visible. In the meanwhile, apparently another crop of pycnidia develops rapidly, and in a few weeks begins discharging spores in large numbers.

This succession of crops continues indefinitely, but under ordinary conditions these fruiting bodies do not appear to be quite as numerous in midsummer or late summer as in the spring or fall.

Usually a number of saprophytic fungi develop in dead bark along with *Phomopsis citri*. Among these, *Colletotrichum*, *Diplodia*, and *Phoma* species are most frequently observed.

#### DISSEMINATION OF THE CAUSAL ORGANISM

From the general structure of the fruiting body and the periods of normal spore discharge, together with the localization of the disease with respect to foci of infection, it would seem that *Phomopsis citri* is disseminated principally through rain drip, rain spatter, driving rain, or dew drip. Spore emergence is plainly visible in rainy periods, but in dry weather it is not apparent, if it occurs at all.

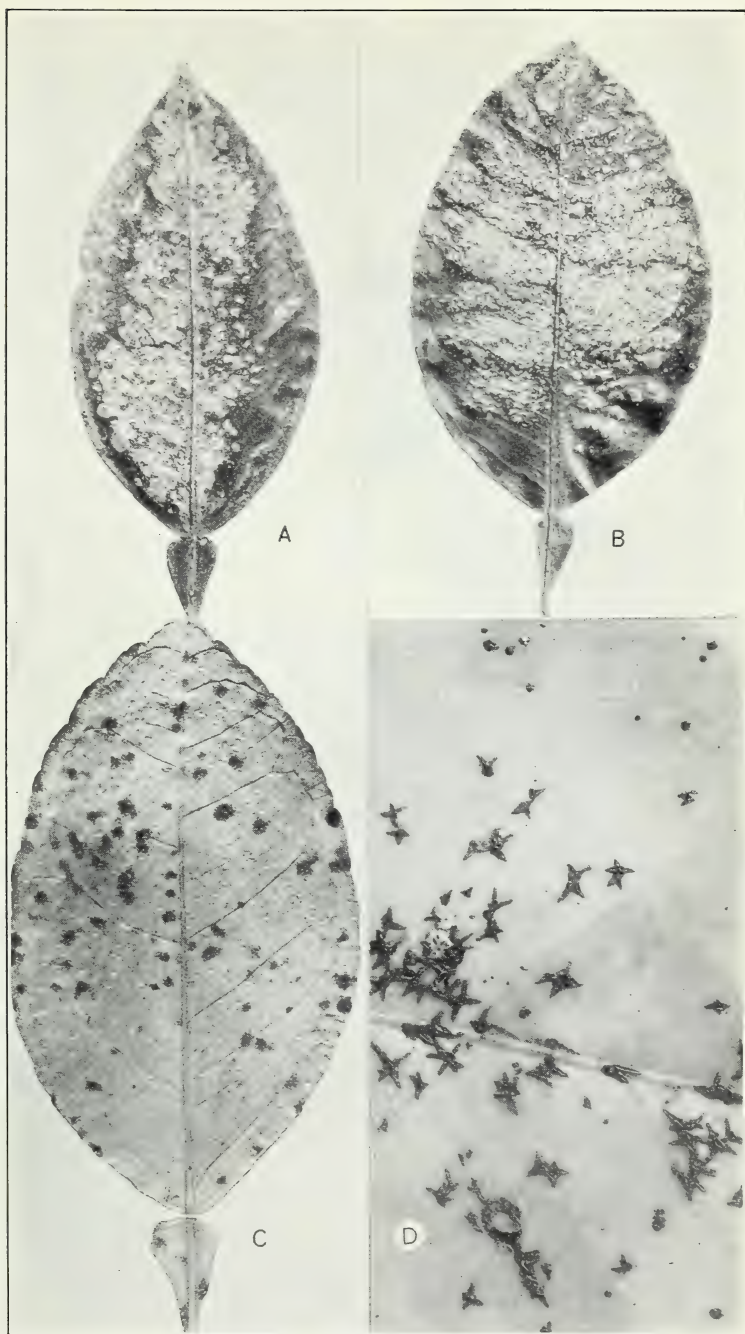
The relative absence of melanose from the tops of trees, as well as from the outermost parts that are not subjected to trickling or spattering water from infected twigs above, and the great abundance of melanose within the head of the tree and on those outer parts that are wet with drippings from dead parts above (pl. 9, C) are strong evidence that the causal organism is water borne. Except in periods of hurricanes and gales there is little or nothing to suggest that spore-laden water is blown any great distance. This is indicated by the fact that in mixed plantings of old, melanose-harboring trees with young resets melanose normally occurs in abundance on the old trees and to a considerable degree on the young trees within 15 to 20 feet of the source of infection, but the young trees 30 or more feet away seldom have melanose markings.

Insects are doubtless capable of accidentally transporting spores of the causal organism, but no evidence has been found to indicate that such animals are importantly concerned in disseminating *P. citri*. This may be accounted for in a measure by the fact that spore-bearing parts of the host seldom attract the common insects of a citrus grove.

In order to determine whether prunings on the ground are a source of melanose infection, prunings of dead or weakened twigs and limbs were left under trees throughout the season of infection, and although the melanose fungus may develop abundantly on these pruned parts, no appreciable increase in melanose was observed on these trees in excess of what developed on pruned trees from which the prunings were hauled away. Even those tender parts within a foot or less above piles of prunings failed to develop melanose in greater quantity than similarly situated parts on check trees. This test was conducted over a period of three years.

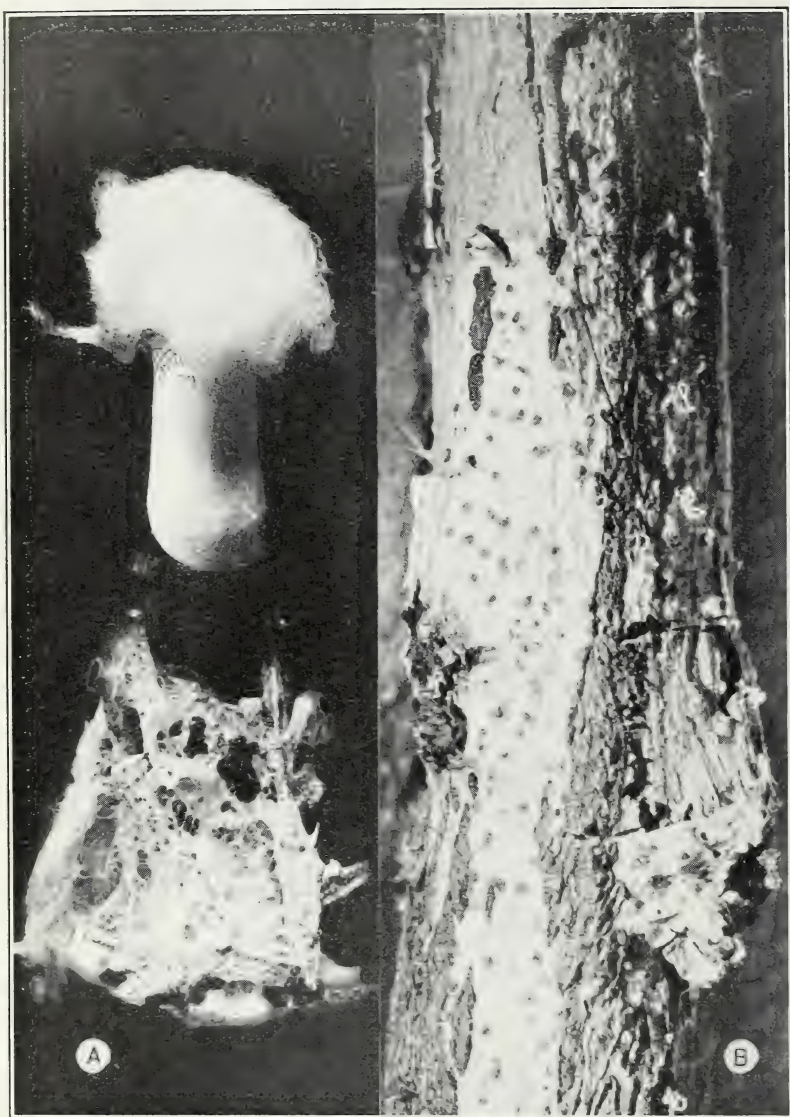
Attempts have been made repeatedly to trap spores in Petri dishes from air currents during fair weather and occasionally during inclement periods. The traps were placed at regular stations in old seedling plantings where there was an abundance of melanose, and exposures were made for varying lengths of time at intervals throughout the day and season. In no case were viable *Phomopsis* spores trapped.





#### MARKINGS FOUND ON GRAPEFRUIT LEAVES

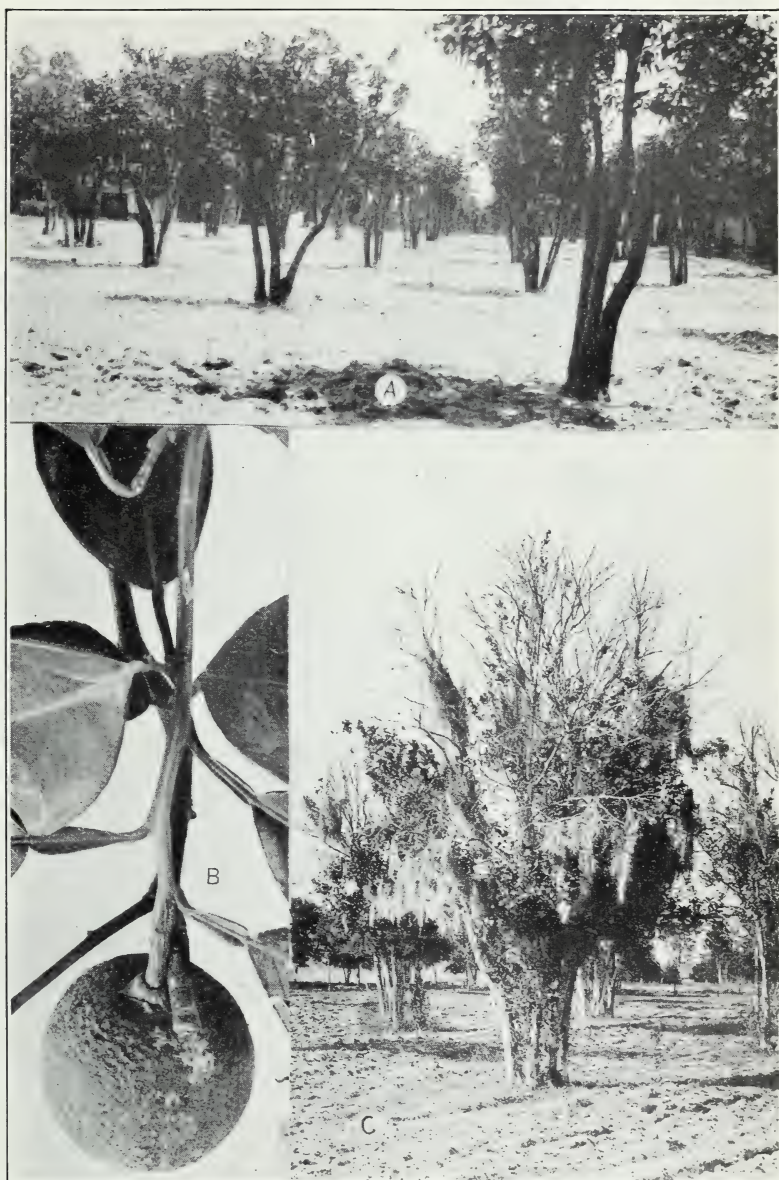
- A and B, Typical Bordeaux-oil spray burn on grapefruit leaves. This injury occurs rarely, and hence is of minor importance.
- C, So-called "black melanose," a leaf injury of uncertain causation which must not be confused with true melanose.
- D, "Star melanose" in enlarged view (X 3). This is probably a form of Bordeaux-spray injury.



PHOMOPSIS GROWING ON PIGEON PEA AND IN BARK

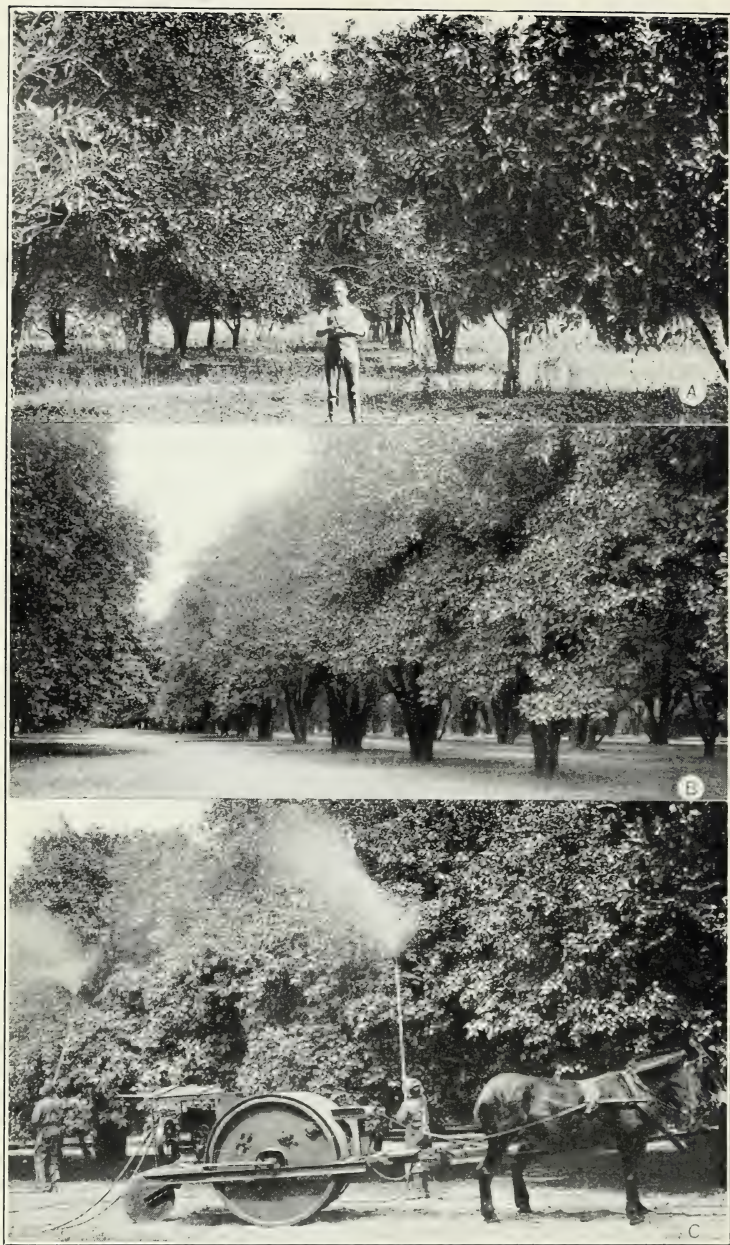
- A, Pure culture of *Phomopsis citri* growing and fruiting on pigeon-pea stems. Most of the spores used in inoculation tests were grown in this manner  
 B, Enlarged view of *Phomopsis* pycnidia developing in bark. Note the white, irregularly coiled spore tendrils showing against the darker background on the right





#### FREEZING EFFECTS ON SEEDLING ORANGE TREES

- A, Seedling orange trees deheaded after the freeze of February, 1917. Photographed in the fall of 1917. As a result of this severe cutting out of deadwood, melanose did not develop to any appreciable extent that year, and the trees were producing normal crops in a few years.
- B, Small dead twig as a source of melanose infection on young orange. It is almost impossible to remove twigs of this size in pruning operations.
- C, Seedling orange trees severely killed back by the freeze of February, 1917, left unpruned and with little care for several years. Had these trees been deheaded and given proper attention they would have recovered in two or three years.



### MELANOSE CONTROL EXPERIMENTS IN SEEDLING ORANGE GROVES

- A, Seedling orange grove in which pruning and spraying experiments were carried on. Considerable deadwood was present
- B, A grove of very large seedling orange trees in which satisfactory control of melanose was obtained by a single application of Bordeaux-oil emulsion made before the onset of the May rains, despite the fact that the size of the trees makes thorough spraying difficult
- C, A dense seedling orange grove in which melanose was successfully controlled with one application of Bordeaux-oil emulsion



Viewed over a period of eight years' observation and study, it seems apparent that dew drip and rain drip or rain spatter from dead parts in the tree are the important means of dissemination of the causal organism.

#### CONDITIONS FAVORING INFECTION

Melanose infections are likely to occur in Florida at any season of the year when rainy periods coincide with the time when leaves, twigs, or fruits are in a susceptible condition. Scattering citrus-melanose scars have been observed on several occasions on flushes of growth developing in winter and repeatedly on those expanding in early spring, but for all practical purposes melanose is not likely to occur in quantity on the early spring flush. Almost every year rainy periods occur in March well after most of the spring vegetative flush has hardened, but long before the fruit has become resistant. When these March rains occur a moderate amount of fruit and flower infection (pl. 1, C) almost invariably takes place, but ordinarily the principal infections seldom occur before May, April being one of the driest months of the year. Along with the May rains come severe melanose outbreaks on the fruit, followed by later outbreaks on the second flush. Fruit setting in May or June is very likely to have melanose markings, although as a general rule spring-bloom fruit in central Florida has melanose worse than summer-bloom fruit. The vegetative flush which expands in late summer or early fall is in general more severely blemished by this disease than any other growth.

Melanose outbreaks vary from season to season and from locality to locality, not only in the cumulative severity of the disease for the season, but also in the severity of the onset of the disease. In certain years the heaviest infections may occur at the very beginning of the rainy periods, especially if the rains continue for 36 to 48 hours without enough interruption to permit the tree to dry off completely. At other times they do not take place until weather conditions have been apparently favorable for infection for 10 days or two weeks.

Ordinarily melanose infection on leaves and twigs does not occur in sufficient proportions to justify special protective measures; however, occasionally there are notable and extreme exceptions. These exceptions have been repeatedly observed in groves receiving good care and frequent pruning, as well as in indifferently cared for properties.

Although outbreaks of citrus melanose appear to be erratic, they are far more uniform than citrus scab, and it frequently happens that within a given locality of a dozen or more groves, all receiving essentially the same care and with approximately the same topography, citrus-melanose outbreaks vary from slight to very severe. It sometimes happens that general grove practices, exclusive of spraying operations, that are intended to keep trees in a vigorous condition and commercially free of deadwood seem actually to promote melanose outbreaks. This has been noted in a number of groves or portions of groves which received several prunings irrespective of whether only

dead and weakened twigs and limbs were removed or whether rather heavy pruning was resorted to.

Citrus melanose occurs on trees of all ages, but more freely in 8 to 10 year old or older plantings. Even in bearing trees less than 10 or 12 years old the disease is not likely to be of consequence if the trees have been kept in a vigorous condition, free of scale insects, and relatively free of deadwood. The damage done by melanose to trees of nonbearing age, including nursery stock, is of negligible importance except following freezes severe enough to produce an accumulation of deadwood or where dead pruning stubs above the buds have become sources of infection.

Ordinarily melanose increases progressively with the advancing age of the planting, and in general those conditions which promote the accumulation of deadwood may be considered predisposing factors to melanose development.

When the leaf or twig is severely infected, complete defoliation of the twig occurs at about the time the leaf should normally develop the deep-green color of maturity. Twigs that bear these severely infected leaves are almost sure to die before it is time for the succeeding flush to come out, and those twigs that have died recently are sources of melanose infection later. Defoliation and blighting are usually worse on the inside of the tree than on the outside and invariably worse on the lower portions of the tree.

#### MICROSCOPIC STUDIES OF MELANOSE MARKINGS

Microscopic examinations of melanose markings have been made by Swingle and Webber (21), Floyd (10), and Stevens (20) without finding consistent evidence of parasitic invaders.

The writers also have made careful examinations of affected parts at intervals throughout the progress of this work. Free-hand and freezing microtome sections and paraffin sections, stained and treated in the usual manner, were studied. Examinations were made of old (pl. 3, A) and young lesions, and in addition especially careful examinations were made of young leaf tissues of the kumquat, a very susceptible species, that had been in contact with spore suspensions of the causal organisms under good conditions for infection for 48 hours, a sufficient length of time to produce melanose markings.

The host tissue of recently inoculated parts gave pronounced evidence of having been killed for a depth of three or four cells, presumably by the melanose fungus, but in none of the microscopic sections of young tissue was there evidence of fungal invasion. In some of the sections of old tissues there was very occasional evidence of fungal hyphæ. This invader was possibly of a saprophytic nature.

#### REISOLATION OF CAUSAL ORGANISM

At intervals throughout the progress of this work on citrus melanose many systematic attempts were made to recover the causal organism from melanose blemishes. Both leaf and fruit tissues were cultured, and not only were old, rough melanose lesions used in these tests but also young, almost invisible spots and even inoculated parts that had not become visible. In some cases the surfaces of the affected parts were washed with common disinfecting agents, such



as ethyl alcohol, ether, acetic acid, mercuric chloride, hydrogen peroxide, and in many instances the parts to be cultured were washed in alcohol or ether for a second or two and transferred to mercuric chloride for varying lengths of time and rinsed in sterile tap water. Whenever fresh hydrogen peroxide was used, washing of the sterilized parts was dispensed with; however, many cultures were attempted without the use of surface disinfection. The sterilizing agents were used with caution to prevent the chance of killing the causal organism within the host if it were present. Fully 1,000 attempts were made, but not in a single instance was the causal organism recovered. On the other hand, this fungus can be cultured readily from dead citrus twigs and from citrus fruits affected with stem-end rot, as well as from the surface of normal tissue.

#### GROWTH ON ARTIFICIAL MEDIA

*Phomopsis citri* grows on a rather wide range of culture media, but on some the mycelial growth is sparse, whereas on others it is abundant. The media used most often in these studies have been corn-meal agar, potato-dextrose agar, steamed stems of pigeon pea (*Cajanus indicus*), sweet clover, grapefruit, and sweet potato.

On corn-meal agar the fungus makes a rapid white growth, mostly a sparse mat on the surface with a small amount of aerial mycelium. Though not abundant, sporulation on this medium occurs within one to four weeks in quantities satisfactory enough for general use.

On potato-dextrose agar there is an abundant, rapid mycelial growth, at first snowy white, darkening somewhat with age; aerial parts fairly abundant and usually fluffy, but at times compact. It sporulates sparsely on potato-dextrose agar, and in many cultures pycnidia fail to develop.

On beef-peptone agar the mycelial growth during the first week or 10 days is very similar in quantity to but somewhat more velvety in appearance than growth on potato-dextrose agar.

On steamed stems of sweet potato, pigeon pea, sweet clover, and grapefruit *P. citri* makes a rapid mycelial growth, and in a short time the stems are covered with white mycelium. At first the growth is flocculent, but as the stems dry the mycelium usually becomes compact, and within 10 days or two weeks at favorable temperatures distinct evidence of pycnidial development is seen. Later these pycnidia become more erumpent (pl. 8, A), and still later cream-colored spore tendrils emerge (pl. 8, B). Ordinarily from two to six weeks are required to develop a crop of spores at room temperature.

In addition to the above-mentioned media *P. citri* grows abundantly on such media as Blakeslee's agar, Leonian's agar, carrot agar, peptone-dextrose agar, potato hard agar, prune agar, and shredded-wheat biscuits. It grows sparsely on beech-twigg agar, Cohn's agar, Czapeck's agar, Ferni's agar, and Uschinsky agar. The organism has also been grown on broths of prune, peach, sweet potato, tomato, potato, orange twigs, and apple twigs.

The rate of growth of this fungus varies considerably, depending upon the medium. On certain media the rate of growth is at first slow for a few days and later is very rapid, but for the most part it may be considered a fairly rapid grower.

## TEMPERATURE RELATIONS

Fawcett (8) has shown that the rate of mycelial growth of *Phomopsis citri* on artificial media is affected markedly by temperatures above and below the optimum for this organism. In general the maximum growth was obtained at temperatures around 27.5° C.

In order to determine the effect of temperatures upon spore production, cultures of *P. citri* on sweet-clover stems were placed in temperature compartments held at fairly definite maintained temperatures for 75 days and examinations made at frequent intervals for spore production.

At temperatures of 2° C. (35.6° F.), 4° C. (39.2° F.), 6° C. (42.8° F.), and 8° C. (46.4° F.), sporulation did not take place during the period under observation. Sporulation occurred at 10° C. (50° F.) in 33 days, at 12° C. (53.6° F.) in 30 days, at 14° C. (57.2° F.) in 24 days, at 16° C. (60.8° F.) in 18 days, at 20° C. (68° F.) in 14 days, and 25° C. (77° F.) in 30 days; but at 28° C. (82.4° F.) and 33° C., respectively, sporulation had not taken place in 75 days.

Thus it is evident that spore production was most rapid at about 20° C. The length of time required for this increased gradually with a gradual reduction of temperature, but the necessary time increased rapidly as the temperature was raised slightly above its optimum, showing that spore development is greatly retarded if not absolutely inhibited at 8° above optimum and 12° below optimum. The 20° C. (68° F.) optimum for spore production is quite distinct from the 27.5° C. (81.5° F.) optimum for vegetative growth.

## SPORE GERMINATION

Spore germination tests were also made at a number of temperatures. From pure cultures of *P. citri* growing on sweet-clover stems, fresh viable spores were placed on microscope slides, covered with plain agar, and put in Petri dishes. These slides were held in incubators and microscopic examinations made at frequent intervals.

Spore germination was found to be most rapid at temperatures ranging from 20° C. (68° F.) to 27° C. (80.6° F.). Good germination had taken place within this temperature range in from 18 to 20 hours. At a temperature of 33° C. (91.4° F.) and at 16° (60.8° F.) or below germination was greatly retarded.

## INFECTION AT MAINTAINED TEMPERATURES

In order to determine the temperature range over which infection takes place, a series of inoculation experiments was conducted on the leaves of potted grapefruit seedlings. These plants were inoculated by saturating absorbent cotton with water rendered cloudy from heavy charges of viable spores of *P. citri* grown in pure culture. Swabs of this wetted cotton were placed on the parts to be infected and then wrapped in paraffin paper. The plants were then placed immediately in incubators for 48 hours, removed from the compartments, paper and swabs removed, washed for one minute in a 5 per cent solution cresol compound or in a 1 to 1,000 mercuric-chloride solution, rinsed in running water for two minutes, and placed under a slat shade to await developments. The progressive increases in



intensity of the outbreaks expressed on a percentage basis are shown in Figure 2.

Figure 2 gives marked evidence of the effect of several maintained temperatures upon infection where the plants were subjected to the inoculum for 48 hours and then surface disinfected. At 15° C. (59° F.) and 30° C. (86° F.) the infection became visible on the sixth day and reached 100 per cent on the twelfth and thirteenth days, respectively. The plants exposed at 20° C. (68° F.) and 25° C. (77° F.) did not show signs of infection until the eighth day and reached 100 per cent on the thirteenth and fourteenth days, respectively, whereas those exposed at 10° C. (50° F.) showed no signs of infection until the ninth day and reached only 56 per cent on the thirteenth day. The degree of infection obtained is indicated on the curve for each temperature.

From these tests it is seen that melanose infection may occur at temperatures as low as 10° C. in 48 hours as well as at 30°. The rate of development and the severity of outbreak of the disease does not appear to be very different at temperatures within the range of these tests. A 5 per cent cresol solution proved to be equally as effective as mercuric chloride for disinfecting surfaces of citrus leaves.

In order to determine further the effect of several temperatures and both shorter and longer exposures upon infection by the melanose fungus, a series of holding tests was run, using potted sour orange and grapefruit plants held at definite maintained temperatures continuously during the infection period. These plants were inoculated by the swab method and placed in various compartments for varying lengths of time. Later they were taken from these compartments, swabs removed, leaves immersed in a 1 to 1,000 corrosive-sublimate solution or 5 per cent solution cresol compound for one minute, rinsed in running tap water, and transferred to slat shade to await developments. Final readings were made three weeks later, and the findings are given in Table 2.

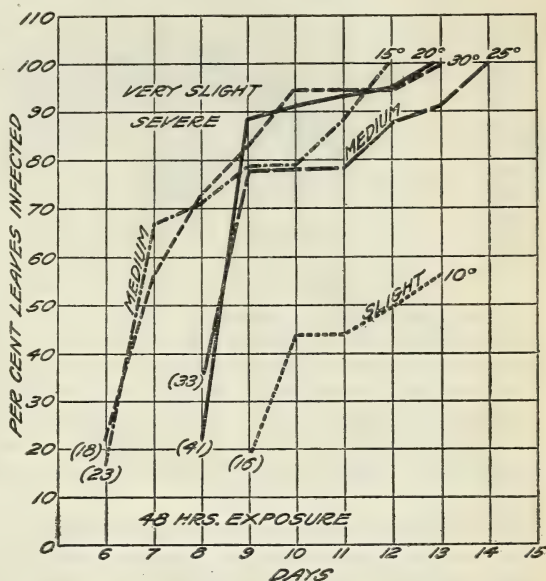


FIG. 2.—Percentages of grapefruit leaves developing melanose infection after being inoculated and incubated for 48 hours at various maintained temperatures (10°, 15°, 20°, 25°, and 30° C.), and then held for 15 days under equal conditions favorable for melanose infection. The number of grapefruit plants in each lot is indicated by numbers in parentheses, and the average intensity of infection as varying from very slight to severe.

TABLE 2.—Effect of definite maintained temperatures for various periods on infection and incubation of *Phomopsis citri* on grapefruit leaves at the end of a 3-week holding period

Time exposed to inoculum	Percentage and character of melanose infection				
	10° C.	15° C.	20° C.	25° C.	30° C.
8 hours	0	0	0	0	0
10 hours	0	0	0	0	0
12 hours	0	15, very slight	67, slight	100, very slight	66, very slight
14 hours	0	91, very slight	100, very slight	100, slight	100, very slight
16 hours	0	100, very slight	100, medium	100, medium	Do.
18 hours		100, medium	100, severe		
20 hours	33, very slight	do	100, medium	100, medium	Do.
24 hours	20, very slight		do	do	100, medium
28 hours	do		100, severe	100, severe	Do.
30 hours	100, slight				
36 hours	do				
42 hours	do				
48 hours	do				
72 hours	100, medium				
90 hours	86, medium				

From the data presented in Table 2 it is seen that infection took place at the four higher temperatures in 12 hours. At the lower temperature longer exposures were necessary to cause infection. As much infection occurred in 12 hours at 25° C. and in 14 hours at 20° as in 30 hours at 10°. Thus it seems possible that natural infection may take place in as short a time as 12 hours if conditions are favorable. Sour-orange leaves were as severely infected as the grapefruit.

In order to determine the incubation period for melanose on plants held at definite maintained temperatures, five series of holding tests were conducted on potted grapefruit plants inoculated, placed immediately in the temperature compartments, and held there until the disease became visible. The results of these holding tests are shown in Figure 3. These show that maintained temperatures in the neighborhood of from 20° to 25° C. are optimum for melanose infection on leaves, not only with respect to promptness of development but also to severity of infection. At 25° the development was somewhat slower, but approximately of equal intensity. At 30° it was slower than at 25° and of less severity; at 15° the rate of development was still slower, resulting in a slight degree of infection. At 10° the development of the disease was very slow and resulted in a very slight infection.

#### OTHER INOCULATION TESTS ON POTTED CITRUS PLANTS

A series of plants was inoculated in the usual manner, placed in an incubator, held at 25° C., and kept there for varying lengths of time, when the plants were removed without disinfecting to the relatively dry slat-shade house. Plants which were held at this temperature for 20, 54, 78, 102, 126, 154, and 174 hours, respectively, showed initial signs of infection in from 5 to 11 days from inoculations. The degree of infection ranged from very slight to medium. This indicates that, under natural conditions with the pathogen present, infection may be expected to occur if the suscepti-



ble parts are moist for 20 hours or more. This is corroborated by general field observations as well as by the results presented in Table 2.

Another series was run to determine the effect of various temperatures upon the infecting power of spores of *Phomopsis citri* in suspension. Spore suspensions were held at 10°, 20°, and 30° C., respectively, and inoculations were made under natural conditions of temperature at the expiration of 8, 24, 32, 48, 72, and 96 hours, respectively. Inoculum held at 10° C. caused infections that for the most part were of a slight degree; that held at 20° caused infection ranging from slight to very severe, averaging a medium degree; and that held at 30° produced 100 per cent infection, ranging from very slight to very severe, averaging slight. Invariably the lightest infection occurred on plants that were inoculated with spore suspensions 72 hours old or older, whereas the heaviest infection developed

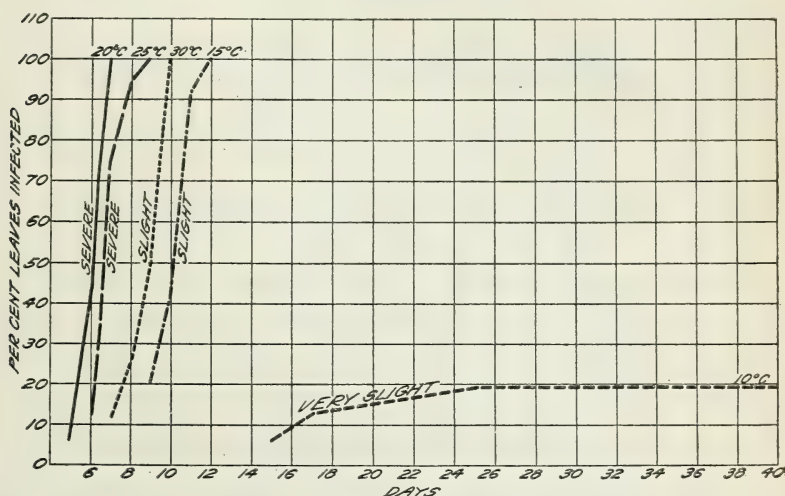


FIG. 3.—Percentage of grapefruit leaves developing melanose infection after being inoculated and held for 40 days at various maintained temperatures from 10° to 30° C. The average intensity of infection for each set of plants is indicated as varying from very slight to severe

from inoculum held 24 hours or less. For checks the plants were inoculated with fresh inoculum that resulted in very severe infection.

Still another series was run in which the plants were inoculated with fresh viable spores; placed in compartments at 10°, 15°, and 20° C.; left there for 16, 24, 40, 48, 72, 96, 120, 144, and 168 hours, respectively; removed and placed in the summer sun for two hours, where they dried before being returned to the same compartments without being washed with a disinfectant. On all plants irrespective of temperature the degree of infection ranged from medium to very severe, and infection of like intensity developed on the check plants which were not sunned. These findings seem to indicate that a short sun exposure has little or no effect upon melanose infection when such exposure occurs as much as 16 hours after inoculation.

## INOCULATION TESTS IN GROVES

During the progress of the work covered in this bulletin outdoor inoculation experiments received far more attention than is usually considered necessary, not only because a relatively small amount of work had been done on this phase of the problem previously but also because Koch's postulates, the generally accepted proof of pathogenicity, can not be followed in two of the steps, at the beginning and end of the cycle. Fortunately these are the least important of the four postulates. For these tests a wide range of rutaceous plants growing under natural conditions on the laboratory grounds of the United States Citrus-Disease Field Laboratory at Orlando, Fla., were used.

In general these experiments were designed (1) to gain further evidence on the pathogenicity of *Phomopsis citri*; (2) to determine the host range of the disease and the relative susceptibility of the various species; and (3) to determine the age or size when leaves and fruits of susceptible forms become immune to infection, in order to place the practical control of the disease on a more definite basis.

For the most part pure cultures of the causal organism for inoculation purposes were obtained from fruits suffering from stem-end rot. These cultures were grown at room temperature on corn-meal agar, potato-dextrose agar, stems of pigeon peas, sweet clover, or sweet potatoes, and when sporulation became abundant the media were washed with sterile water in order to get the spores in suspension. Ordinarily the spores were plentiful enough in this wash water to cause it to become distinctly clouded. These same cultures were used repeatedly for spore production until they became contaminated. Absorbent cotton was saturated with this spore-charged water, and liberal portions of the wetted cotton were placed on the parts to be inoculated, wrapped immediately with several layers of paraffin paper, and left on from 24 to 48 hours, depending upon the weather, before the wrappers and cotton swabs were removed. Ordinarily, less than 15 minutes elapsed between washing the spores and applying the inoculum. No other method of inoculation was employed in any of the infection tests.

The inoculations were made under a wide range of conditions of weather and tenderness of host parts. The tests were so extensive and involved such a wide range of hosts that it was obviously impossible to have tests on each host rounded out to the extent that scientific interests may demand, but they do seem sufficiently complete for a practical consideration of the problem.

In addition to the large number of inoculations on the orange and the grapefruit, tests were made on miscellaneous citrus plants. At least 30 inoculations with a liberal number of checks were made on each host. The results of inoculations on rare forms that are not accounted for in the following tables are recorded in Table 1.

There was a pronounced increase in the degree of infection from slight or medium in the early tests to very severe on the later ones, even on the same plant. This increase in intensity of infection is probably due to the use of a thicker swab with heavier spore charges in the later tests than was the case earlier. Such an explanation seems probable, since it is next to impossible to have the spore-laden wash water from cultures charged uniformly, and the natural tend-



ency was toward increasing the concentration of the inoculum. The thickness of the swab probably increased as time went on, permitting a slower drying out of the inoculum, which no doubt aided in accomplishing infection.

## INOCULATIONS ON LEAVES

In Table 3 it is shown that small leaves of the representative varieties of the orange, the grapefruit, the kid-glove oranges, and the lemon are at first very susceptible to melanose infection, and as they enlarge with age they become progressively resistant, reaching practical immunity when they become about 2 inches wide, on an average. It is questionable whether the trace of "infection" that developed on these leaves was true melanose or some injury resulting from the wetted cotton swabs.

TABLE 3.—Combined results of melanose inoculations on citrus leaves at Orlando, Fla., 1919 to 1924

Kind of citrus	Number of varieties tested	Width in sixteenths of an inch	Number of inoculations	Percentage positive	Degree of infection	Water blank checks	
						Number	Percentage positive
Orange	14	0 to 4	384	91	Severe	130	0
		5 to 8	233	74	do		
		9 to 12	407	65	do		
		13 to 16	488	62	Medium		
		17 to 20	462	49	do		
		21 to 24	403	56	Slight	176	0
		25 to 28	238	24	Very slight		
		29 to 32	135	21	Trace		
		Over 32	157	10	do		
		0 to 4	525	90	Very severe		
Grapefruit	11	5 to 8	124	70	do	123	0
		9 to 12	263	66	Severe		
		13 to 16	386	73	do		
		17 to 20	399	64	do		
		21 to 24	445	48	Slight		
		25 to 28	352	42	Very slight	3	0
		29 to 32	251	31	Trace		
		Over 32	435	16	do		
		0 to 4	256	94	Very severe		
		5 to 8	79	52	Severe		
Kid-glove orange group	6	9 to 12	149	37	do	64	0
		13 to 16	223	25	Medium		
		17 to 20	139	22	Slight		
		21 to 24	109	14	Very slight		
		25 to 28	60	15	do		
		29 to 32	31	21	Trace	89	0
		Over 32	7	0	do		
		0 to 4	166	89	Very severe		
		5 to 8	82	41	do		
		9 to 12	103	54	Severe		
Lemon	6	13 to 16	136	49	Medium	54	0
		17 to 20	85	48	do		
		21 to 24	56	43	Slight		
		25 to 28	55	38	Very slight		
		29 to 32	48	31	Trace		
		Over 32	50	24	do		

In addition to the inoculations listed in Table 3, numerous inoculations on leaves of various citrus and related plants, ranging from four-sixteenths to five-sixteenths of an inch in width, were also made.

One hundred and seventy-four inoculations on five varieties of limes gave 91 per cent infection, averaging severe, whereas 75 water blank checks showed no signs of infection.

In the sour-orange group 198 inoculations on five forms gave 82 per cent infection that averaged severe in degree, and the 60 checks failed to become infected.

On 18 hybrid forms, mentioned in Table 1, 636 inoculations gave 90 per cent infection that averaged severe in degree. In general, the degree of infection was worse on the kumquat hybrids and on the faustrimedin than on any other. The 213 water blank checks failed to develop melanose.

One hundred and ninety-two inoculations were made on leaves of six miscellaneous citrus forms—namely, the Nagami, Marumi, and Meiwa kumquats, the Corsican and Etrog citrons, and the calamondin. The kumquats were found to be extremely susceptible, and the others were moderately so. The 78 water blank checks remained free of melanose.

Three hundred and fifteen inoculations were made on the eight noncitrus rutaceous genera mentioned in Table 1. Of these, *Toddalia lanceolata*, *Chalcas exotica*, and *Citropsis schweinfurthii* failed to show signs of infection, and none of the remaining five gave indication of being more susceptible than the orange. The 165 water blank checks failed to develop melanose.

Leaves of the orange and the grapefruit are extremely susceptible when about one-fourth inch (6 millimeters) in width. As they expand they become progressively resistant and acquire practical immunity about the time they toughen, which usually takes place considerably before the leaf takes on a deep-green color. Ordinarily leaves are susceptible to infection for two weeks or less in periods of good growing weather, but for a considerably longer time if the weather is unfavorable to growth. Likewise the width of a leaf at a given age varies greatly, owing to conditions of weather and of tree vigor. Because of this variation the width of leaf does not indicate with definiteness the condition of susceptibility.

#### INOCULATIONS ON FRUITS

Table 4 gives the results of inoculations with spores of *Phomopsis citri* on the fruit of various citrus types.

Table 4 shows that the orange is susceptible to infection until it reaches a diameter of approximately  $1\frac{1}{2}$  inches (38 millimeters), and it also shows that the degree of infection is much more severe on small fruits than on larger ones. All commercial varieties tested were about equally susceptible. The water blank checks show that melanose did not develop on those fruits, indicating that moisture alone is not responsible for melanose lesions.

The grapefruit is susceptible to infection from blossoming time until it reaches a diameter of approximately  $2\frac{1}{2}$  inches (63 millimeters). As is the case with the orange, there is sufficient evidence to conclude that the grapefruit is very susceptible when very small and becomes progressively resistant with increasing size. Again all commercial varieties tested were about equally susceptible. General observations and the results of spraying tests in commercial plantings confirm these findings. The checks on the young named varieties failed to develop melanose markings.

The kumquat, lemon, lime, sour orange, bergamot orange, calamondin, citrangequat, faustrime, limequat, and tangor were all very susceptible to melanose infection, whereas melanose did not develop on the checks.



TABLE 4.—Combined results of melanose inoculations on citrus fruits at Orlando, Fla., 1919 to 1924

Kind of citrus	Number of varieties in the test	Diameter in sixteenths of an inch	Inoculations		Degree of infection	Water blank checks	
			Number	Percentage positive		Number	Percentage positive
Orange.....	7	0 to 4.....	40	92	Severe.....		
		5 to 8.....	275	49	do.....		
		9 to 12.....	202	56	Medium.....		
		13 to 16.....	216	55	do.....		
		17 to 20.....	194	37	Slight to very slight.....		
		21 to 24.....	328	36	Very slight.....	40	0
		25 to 28.....	190	0			
		29 to 32.....	76	0			
		0 to 4.....	130	74	Very severe to severe.....	150	0
Grapefruit.....	7	5 to 8.....	20	75	Severe.....		
		9 to 16.....	277	47	Medium.....	143	0
		17 to 24.....	301	76	do.....	40	0
		25 to 32.....	267	76	do.....	82	0
		33 to 40.....	288	35	Slight.....	10	0
		41 to 48.....	226	0		20	0
Kumquat.....	2	8.....	56	100	Very severe.....	20	0
Lemon.....	2	20 to 24.....	28	100	Slight to very slight.....	2	0
Lime.....	2	10 to 18.....	32	100	Medium.....		
Sour orange.....	2	8 to 10.....	36	100	Severe.....		
Bergamot orange.....	1	8 to 10.....	30	100	do.....		
Calamondin.....	1	10.....	20	100	do.....		
Citrus hybrids.....	4	10 to 12.....	125	100	Very severe to medium.....	33	0

From the inoculation tests reported in this bulletin, together with many careful observations in groves and experimentally sprayed plots, it is evident that the young fruit of the orange is at first very susceptible to infection. It becomes resistant gradually and develops immunity when it has reached about  $1\frac{1}{2}$  inches (38 millimeters) in diameter. The grapefruit starts life considerably more susceptible to infection than the orange, and it, too, becomes progressively resistant as it increases in size, reaching immunity when the fruit is about  $2\frac{1}{2}$  inches (63 millimeters) in diameter.

The calendar date at which spring-bloom fruit reaches these stages of growth varies considerably, not only from year to year but also on the same tree during any given season, because of the lengthy blossoming period extending from February through the greater part of March. However, the majority of the fruit from the spring bloom in the neighborhood of Orlando, Fla., seldom reaches this immune stage of growth before May 20 and rarely later than June 10.

#### MISCELLANEOUS INOCULATIONS

In addition to the foregoing inoculation experiments, tests were made to determine whether or not the mycelium of *Phomopsis citri* could cause infection, and other tests were made with spore suspension of this fungus to determine the effect of spray coatings, boiling of the inoculum, and aging of the inoculum upon subsequent infection. These tests were repeated several times and may be summarized as follows: Fully 200 inoculations were made with the mycelium of *P. citri* on tender orange and grapefruit leaves. In practically every case infection failed to develop, but on a few of the inoculated leaves occasional melanoselike spots appeared. If these spots were

true melanose markings they most likely developed from spores already on the leaves or from stray spores accidentally transferred with the mycelium, otherwise a larger percentage of leaves should have been affected. The water blank checks showed about as much infection as the inoculated plants.

Inoculations with viable spores consistently failed to infect young leaves sprayed with lime-sulphur solution a few hours before the inoculum was applied. Similar inoculum heated to the boiling point failed to cause infection. Inoculum held for 24 hours at room temperature, 24° to 32° C. (75° to 90° F.), gave 100 per cent infection.

In order to determine whether or not ordinary spring-bloom oranges and grapefruit can become infected with melanose during the late summer months, a simple test was made by tying small bundles of dead citrus twigs around the stems of fruit in August and leaving them there until the fruit matured. The dead twigs were especially selected for the presence of large numbers of fruiting bodies of the causal organism. No signs of infection had developed on any of the fruit by midwinter, when most of the grapefruit and some of the oranges were fully mature.

Attempts were also made to infect living twigs at leaf scars with spores of the melanose fungus. Inoculations were made on twigs partially defoliated by applications of oil sprays as well as on twigs on which the usual natural defoliation had occurred. None of these inoculations gave the slightest indication of infection, nor could injury in any way be detected nine months later from the attempted inoculations.

If such infections were to take place in nature there should be abundant and obvious indications of it, since some defoliation occurs regularly after applications of insecticidal oil sprays that are applied at almost any season of the year. In addition to this induced defoliation, a fair amount of foliage drops each spring as the result of natural causes, but no evidence has been gathered to indicate that such living twigs become infected and later die as a result of this infection. On the contrary, there is an abundance of evidence that twigs become infected after the bark has died from one cause or another.

#### SUMMARY OF INOCULATIONS

Summed up, the evidence at hand, together with the results of former investigators, prove beyond a reasonable doubt that melanose is caused by the fungus *Phomopsis citri*. The fruit and leaves of many rutaceous forms, including all citrus varieties grown commercially in Florida, are subject to attack by this fungus. Different maintained temperatures during the infection period and different lengths of exposure to the inoculum have a marked influence on the length of the inoculation period and on the degree of the resulting infection as well. Infection can take place in 12 hours at temperatures ranging from 15° to 30° C. (59° to 86° F.); the greatest degree of infection seems to occur between 20° and 25° C. (68° and 77° F.); temperatures above and below 20° C. (68° F.) seem to prolong the incubation period. Attempts to infect hardened twigs through leaf scars failed. Likewise, negative results were obtained when leaves were inoculated with the mycelium of the melanose fungus.



## EXPERIMENTS FOR MELANOSE CONTROL

As soon as it was learned that melanose was caused by a fungus that sporulates in deadwood only, control measures seemed likely to be simple. It was then thought that pruning away the dead and dying parts of trees would not only be a good general practice for invigorating the tree but would also prevent subsequent melanose outbreaks for one season at least. With this idea in mind Stevens (18) began pruning experiments in 1913 for melanose control. Up to that time melanose was considered by growers generally to be a disease that could not be prevented.

Later the writers conducted spraying experiments for melanose control which gave satisfactory results. More recently trial has been made of fungicides to be applied in the form of dust, but these have not yet proved satisfactory.

During the first few years of the present investigation the data from these control experiments were based on counts of 1,000 fruits graded merely as infected or not infected. The counts were made while the fruit was still on the tree, and all trees in a plot were included. Whenever feasible only fruit from the lower portions of the tree was considered, in order that the data would be from fruits that had a good chance to become infected.

Beginning with the tests of 1922, a study of the actual control of melanose more comprehensive than in former years was attempted by grading and dividing the fruit into six classes with respect to melanose infections. Representative fruits of the various grades were photographed and these photographs used as a standard for comparison (pl. 4) in these and subsequent counts. Fruit with "slight infection" is usually packed in the first grade, and in many houses fruit with as much as "medium infection" would be put in the first grade.

The three principal control measures—namely, pruning, dusting, and spraying—will be discussed in order.

## PRUNING FOR MELANOSE CONTROL

Stevens (18, 19) has shown that melanose can be controlled or materially reduced by thoroughly pruning out all deadwood. His findings have been confirmed in commercial practice by a number of growers almost every year, but in the great majority of cases this method has not given a satisfactory control. Because of this inconsistency in results obtained by commercial citrus growers it was considered advisable to conduct further pruning experiments for melanose control.

An old seedling orange grove at Orlando, Fla. (pl. 10, A), with more than the average amount of deadwood was selected for these experiments. In general this grove is about an average one for its age, and it is fairly representative of a large percentage of the seedling orange groves of the State. Probably 50 per cent of the entire orange crop of Florida is at present produced in seedling groves. Throughout the pruning experiments only dead and weakened parts were removed, and the work was done with far more care and thoroughness than is likely to be the case in commercial work. These experiments were started in January, 1921, when a

block of trees was pruned. Another block was pruned in February, and still another block in March, and so on throughout August of that year.

The following year, 1922, pruning experiments were repeated. A part of each 1921 plot was repruned, and an additional plot was pruned for the first time in 1922. Pruning experiments were again repeated in 1923. That year the test was made in the same grove used in the two preceding years and on trees of a similar character but unpruned within the last six or eight years. They had possibly somewhat less deadwood in them than had those used in previous years.

At the end of each season 1,000 or more fruits from each pruned plot were graded for melanose infection, and for comparison many counts were made of fruit from unpruned trees.

The results as shown in the fruit crops of the second (1922) and third (1923) year are given in Table 5. The first year's results were essentially the same as those of the second.

TABLE 5.—*Results of pruning experiments for citrus melanose control in a seedling orange grove at Orlando, Fla., in 1922 and 1923*

Date pruned	Melanose infection					
	Ab-sent	Very slight	Slight	Med-ium	Severe	Very severe
COUNTED IN 1922						
Jan. 15 to 20, 1921; Apr. 6 to 8, 1922	Per cent 0	Per cent 3	Per cent 9	Per cent 32	Per cent 26	Per cent 30
Feb. 15 to 20, 1921; May 8 to 9, 1922	0	0	1	26	37	36
Mar. 15 to 20, 1921; June 8 to 10, 1922	0	0	1	17	45	37
Apr. 20 to 26, 1921; July 10 to 12, 1922	0	1	1	25	46	27
May 26 to 30, 1921; Aug. 10 to 12, 1922	0	1	19	29	34	17
June 20 to 26, 1921; Sept. 11 to 12, 1922	0	3	33	42	21	1
July 18 to 22, 1921; Oct. 10 to 12, 1922	0	6	31	38	22	3
Jan. 15 to 20, 1921	0	0	5	38	31	26
Feb. 15 to 20, 1921	0	0	6	35	39	20
Mar. 15 to 20, 1921	0	0	12	22	25	41
Apr. 20 to 26, 1921	0	7	26	23	32	12
May 26 to 30, 1921	0	4	38	34	23	1
June 20 to 26, 1921	0	9	31	31	24	5
July 18 to 22, 1921	0	10	29	35	21	5
Aug. 23 to 24, 1921	0	14	18	30	28	10
Apr. 6 to 8, 1922	0	1	7	15	45	32
May 8 to 9, 1922	0	0	3	17	52	28
June 8 to 10, 1922	0	1	5	35	33	26
July 10 to 12, 1922	0	1	4	18	53	24
Aug. 10 to 12, 1922	0	2	25	40	30	3
Sept. 11 to 12, 1922	0	2	15	42	39	2
Oct. 10 to 12, 1922	0	0	12	38	42	8
Average of unpruned checks	0	8	16	22	37	17
COUNTED IN 1923						
March, 1923	0	10	35	44	9	2
April, 1923	0	0	45	36	17	2
May, 1923	0	0	45	28	17	10
June, 1923	0	1	22	37	24	16
Average of unpruned checks	0	0	34	30	28	8

For three consecutive years pruning failed to have any appreciable effect upon melanose outbreaks on seedling oranges in these experimental plots. The slight differences in results obtained are due perhaps more to the general condition of the trees than to the efficacy of pruning as a means of controlling melanose. From these tests it is evident that the removal with reasonable care of dead and weakened parts can not be regarded as a dependable method for melanose



control in seedling orange groves presenting essentially the same conditions as those prevailing in this grove. These pruned trees had a greater vegetative growth than the unpruned ones, and melanose infection was about equal the first season. The second year after pruning, melanose was invariably worse on pruned growth than on unpruned trees, owing to the overstimulating effect of pruning.

#### PRUNING AS A SOURCE OF INFECTION

The removal of prunings from a grove is expensive, especially where the trees are planted so thickly that it is difficult to drive a team through without bruising the fruit or trees. Therefore if prunings can be left to rot where they fall, there would be less general objection to this highly desirable grove practice. With that thought in mind, tests during two seasons were made to determine if prunings left to decay on the ground around orange and grapefruit trees resulted in harmful effects on the tree or the fruit.

After many careful observations the conclusion reached was that prunings on the ground around citrus trees are not a source of appreciable increase of melanose or of *Phomopsis* stem-end rot infection. This deduction seems logical, since the causal organism is spread mostly by rain drip and dew drip.

#### COMMERCIAL PRUNING TESTS

Repeated observations have been made during the last few years on commercial pruning operations in normal bearing groves where in most cases the pruning was done once a year but occasionally twice a year. Almost without exception the pruning out of dead twigs resulted in a more vigorous growth and, some growers thought, in a heavier set of fruit as well, which seemed to make pruning a good investment notwithstanding a usual failure to reduce melanose infection. In only rare instances has there been observed any appreciable reduction in melanose in lightly pruned normal bearing groves, but a marked reduction often follows heavy pruning. On the other hand, melanose sometimes becomes markedly worse in trees pruned lightly than in unpruned trees in the same grove. This usually occurred the year following the pruning.

There are conditions under which pruning for melanose control has been very effective,—namely, in normal, young, nonbearing groves where there is very little deadwood present, and in groves of any age following severe freezes, wherever there is a large increase in the quantity of deadwood. This method of preventing melanose on trees severely killed back was clearly demonstrated in 1917 in many groves throughout the State.\* (Pl. 9, A, B.) In that year in several large groves in Lee County in the southern part of the State, and in others in Putnam County close toward the northern limit of commercial orange growing, pruning was begun within a few weeks after the freeze and kept up until the job was completed, even through late summer and into fall. All of the trees were severely cut back in order to remove the recently killed parts, and in many instances the trees were severely headed in or “deheaded.” Wherever this severe pruning was done in early or mid-spring before much

melanose infection took place, the new growth came out and hardened commercially free of the disease and the later flushes did not develop melanose. On the contrary, those parts of the grove pruned during late spring had an abundance of melanose on the spring flushes and little or none on the growths that developed after pruning. Those parts of the grove pruned in late summer or early fall naturally were about as much injured by melanose as the unpruned groves.

#### COST OF PRUNING

The cost of pruning depends largely upon the thoroughness of the job, but it is commercially impracticable to attempt to remove all of the small twigs, fruit stems, etc., and it is these parts that are fertile sources of infection. The writers have records of some young bearing groves pruned under contract for 20 cents a tree. In large bearing trees under normal conditions of vigor the cost is more because of the slowness of the work. Many growers estimate thorough pruning to cost 50 to 75 cents a tree, depending upon the season of the year and the prevailing cost of labor. Pruning as usually done is a slow, tedious, and costly operation and seems to be an unsatisfactory and ineffective means of preventing melanose outbreaks in average groves in Florida.

#### PRUNING INEFFECTIVE

Summed up, the attempt to control melanose by pruning out all deadwood, the source of infection, has generally proved ineffective and excessively expensive except where trees required severe cutting back or deheading.

#### DUSTING FOR MELANOSE CONTROL

Within the last few years the application of fungicides in the form of very fine dust has attracted considerable attention from owners of large acreages. This method of applying fungicides is a rapid one, so much so that two men and a tractor or a fast-walking team can easily dust 40 acres of grove in a day. Little or no more time is required for dusting a grove of large trees than for a similar acreage of small ones. Because of the one great practical advantage of dusting over spraying—namely, speed—considerable dusting has been done in Florida citrus groves within the last few years. Many observations have been made in commercially dusted groves for comparison with results obtained from dusts applied experimentally by the Bureau of Plant Industry for melanose control.

In 1922 a block of seedling orange trees was dusted very thoroughly twice for melanose control. The first application was made April 29, before infection had taken place, and the second on May 22. Both Bordeaux dust containing 12 per cent metallic copper and 15-85 copper-lime dust were used. The following year (1923) 20-80 copper-lime dust was used as well as sulphur dusts. Again the trees were dusted with great thoroughness and in time to control melanose. In 1924 six plots in the same grove were dusted from one to three times in late April and early May, well in advance of much melanose infection. The results of dusting experiments for melanose control in 1922, 1923, and 1924 are given in Table 6.



TABLE 6.—*Results of experimental dusting of orange trees for melanose control in Florida in 1922, 1923, and 1924*

Material used	Number of application	Date applied	Melanose infection					
			Absent	Very slight	Slight	Medium	Severe	Very severe
Copper-lime dust, 15-85.	2	Apr. 29, May 22, 1922.	Per cent 0	Per cent 8	Per cent 17	Per cent 19	Per cent 23	Per cent 28
Bordeaux dust, 12 per cent copper.	2	do.	0	6	16	54	15	9
3-4-50 Bordeaux-oil emulsion spray.	1	May 1, 1922.	62	24	12	2	0	0
Average of unsprayed plots.	0	No treatment.	0	2	17	25	33	23
Dusting sulphur.	2	May 4 and 11, 1923.	0	1	53	35	11	0
Coarse flour sulphur.	2	do.	0	1	39	45	12	3
Copper-lime dust.	3	May 4, 11, 21, 1923.	0	23	48	15	7	1
Do.	4	May 4, 11, 21, 28, 1923.	0	47	46	7	0	0
Do.	2	May 4 and 11, 1923.	0	44	34	20	1	1
Do.	3	May 4, 11, 28, 1923.	0	65	28	5	1	1
3-4-50 Bordeaux-oil emulsion spray.	1	May 4, 1923.	33	48	15	3	1	0
Average of unsprayed plots.	0	No treatment.	0	13	41	35	8	3
Copper-lime dust, 20-80.	1	Apr. 29, 1924.	0	0	6	36	40	18
Do.	2	Apr. 29, May 6, 1924.	0	0	16	41	34	9
Do.	3	Apr. 29, May 6, 13, 1924.	0	4	12	37	35	12
Do.	2	May 6 and 13, 1924.	0	6	26	45	17	6
Do.	1	May 6, 1924.	0	7	32	34	18	9
Do.	1	May 13, 1924.	0	5	17	26	35	17
3-4-50 Bordeaux-oil emulsion spray.	1	May 7, 1924.	70	15	11	4	0	0
Average of unsprayed plots.	0	No treatment.	1	10	23	36	20	10

The data show that the copper-lime dust, the Bordeaux dust, and sulphur dust did not give any appreciable control of the disease in 1922, whereas Bordeaux sprays applied at about the same time in the same grove gave good control. In 1923 two applications of sulphur dusts failed to reduce melanose, but several applications of copper-lime dust did apparently reduce somewhat the severity of the outbreak. Single applications of Bordeaux-oil emulsion spray made in late April or early May gave a much better control of the disease than did as many as four dustings.

The results obtained from dusting experiments in 1924 were certainly no more favorable than in any of the preceding years.

Growers generally, so far as the writers' observations go, have obtained somewhat similar results with dusts for melanose control. In one or two instances from fair to good control appears to have resulted from dusting, but in the great majority of cases the disease has not been materially reduced. In the few cases where good results seemed to have been obtained from a reasonable number of applications of copper-lime dust no undusted trees were left as checks, and it is questionable whether the relative absence of the disease was due to the fungicidal effects of the dust or to a failure of the disease to develop even on undusted parts. Further, insect pests and mites usually do not become appreciably more plentiful on dusted plots during sum-

mer months than on undusted trees, indicating that the fungicidal effects of the dust had largely disappeared before entomogenous fungi began operations in quantity.

Experimental and commercial dusting with the present-day dusts over a period of three years has not given satisfactory melanose control. This is probably due to insufficient adhesiveness.

#### COST OF DUSTING

An average-sized orange or grapefruit tree, 15 or 20 years old, requires approximately 1 pound of dust for each application, and if it be assumed that two or three properly timed applications of dust might be as effective against melanose as one spray application, then a tree will require 2 or 3 pounds of dust. At the prevailing prices of copper dusts in wholesale lots during the last three years, the material alone would cost from 20 to 30 cents per tree. The cost of application is only a small fraction of a cent per tree.

#### DUSTING UNSATISFACTORY

To sum up, dust, either copper or sulphur mixtures, with but a few exceptions, have failed to give satisfactory control of melanose in Florida. In general the results have been so disappointing that this method does not appear worthy of trial on a large scale. The cost of application on the tree basis is equal to or greater than that of standard sprays of proved efficiency.

#### SPRAYING FOR MELANOSE CONTROL

As early as 1896 Swingle and Webber (21) showed that citrus melanose outbreaks could be greatly reduced by applications of copper sprays, and for the control of this disease they gave a tentative spray schedule based on their experimental results. In spite of the fact that it had been shown that this disease could be controlled by applications of sprays, few if any growers attempted to do so, because the injurious effects resulting from an abnormally great increase in scale insects following applications of copper sprays were usually more damaging to the tree and fruit than the severest outbreak of melanose.

With the introduction of Bordeaux-oil emulsion spray, spraying for the prevention of citrus diseases has been put on a far different basis and the former objections to the use of Bordeaux mixture have been largely overcome. Considerable spraying is now being done for melanose control.

The spraying experiments reported in this bulletin are extensive, and records of numerous spraying tests on a commercial basis are also given in order that general conclusions of a dependable nature may be correctly reached, particularly from the viewpoint of the commercial grower. The disease varies in intensity from one grove to another, even from tree to tree, owing to terrain, condition of the tree, stage of growth at time of infection periods, and many other causes. Such factors become more apparent with an increased experience with the disease. Because of this lack of uniformity in melanose outbreaks the control results necessarily must vary considerably, and in deducing conclusions from spraying operations



only general conclusions should be attempted at this stage of the development of melanose control. To take any one plot or even any one grove as a standard for comparison may prove misleading, but if the plots and groves are taken collectively the results point in the same general direction each year.

#### SPRAYING EXPERIMENTS IN 1917

The attempt to control melanose by sprays, made in 1917 by the Office of Fruit Diseases, following the freeze of February of that year, is the second recorded effort to control melanose by sprays. A small preliminary spraying experiment was conducted at Orlando, Fla., in an old seedling orange grove that had been severely damaged by the freeze. Bordeaux mixture, ammoniacal solution of copper carbonate, commercial lime-sulphur solution, and self-boiled lime-sulphur solution were used. The first application was made on April 12 and 13, the second on April 26, 27, and 28, and the third on May 10 and 11, followed by an application of insecticide on June 12 to prevent excessive scale increase.

This grove, previously damaged by foot rot, was so severely injured by the freeze that there was little or no bearing wood left, and the small crop that did set developed from late May and June bloom. All of the deadwood was left in the trees as a source of infection for the new succulent growth which came on during the spring and summer months. The sprayings were not carried on through the summer as had been planned, therefore the applications that were put on gave only a partial protection to the vegetative growth and little or no protection to the fruit that set in June. The fruits on all of these sprayed plots were severely affected by either rust-mite russet or the "ammoniation" that frequently develops on trees suffering with foot rot; these conditions were so prevalent that it was practically impossible to determine with accuracy the degree of melanose infection on fruits.

The results of these preliminary tests showed that there was a marked reduction of melanose on vegetative parts on the plots that received Bordeaux mixture, but this was confined to the growth that took place during April and May. Melanose control on the plots sprayed with ammoniacal solution of copper carbonate was decidedly less pronounced, whereas those that received lime-sulphur solution had only a slight control if any at all. The plots that received one or two applications of ammoniacal solution of copper carbonate followed by one or two applications of sulphur compound were about as severely injured as the plots that received three applications of lime-sulphur solution. "Ammoniation" was much more prevalent on the checks and sulphur-sprayed trees than on those that received copper sprays.

The rainy periods at Orlando, Fla., during the spring months of 1917 are shown in Figure 4. From this it is evident that there was not sufficient rainy weather during March to result in much melanose infection, and, as April was very dry, little or no infection took place. May also was very dry, but a moderate amount of infection developed during the first 10 days of the month. Beginning about the middle of June, the rainy season was accompanied by heavy melanose outbreaks.

## SPRAYING EXPERIMENTS IN 1920

Spraying experiments for the control of melanose were again taken up in 1920 in a mixed orange and grapefruit grove at Orlando, Fla., and in another grove at Winter Park, while a large commercial citrus property in Marion County, another in Polk County, and a third in Pinellas County were sprayed by the owners with Bordeaux-oil emulsion for the control of this disease. There was an abundance of deadwood in all these groves, and melanose had been a major factor for many years. The results obtained in the commercial properties will be discussed further on. Beginning this year and continuing throughout the following seasons reported herein, the sprayed plots were enlarged to the extent that each plot consisted of from 10 to 20 or more trees and required from 150 to

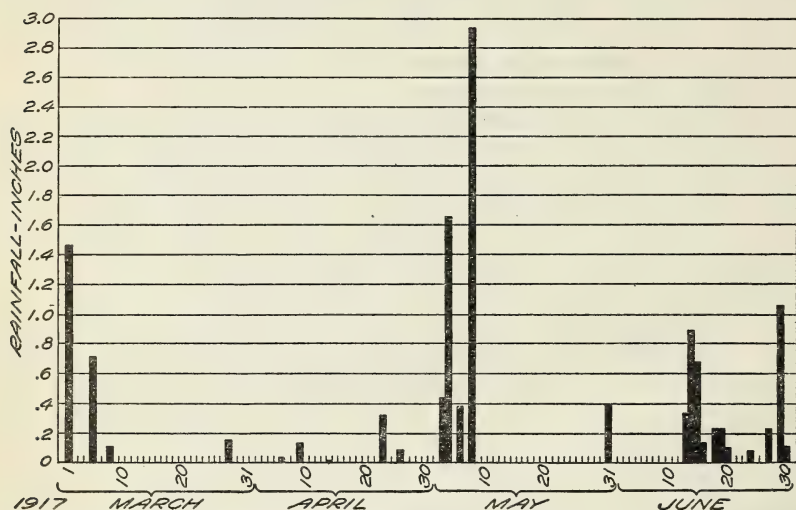


FIG. 4.—Rainfall (in inches) at Orlando, Fla., during March, April, May, and June, 1917

200 gallons of spray for each application. This was done to approximate commercial spraying more nearly and to overcome individual differences that might appear on a few trees. The pressure used ranged from 250 to 400 pounds, the higher pressure being used in the last two or three years because it became evident that more thorough work was done with the increased pressure.

The groves at Orlando and Winter Park under experimental tests were sprayed in accordance with the data and with the results shown in Table 7.

It will be seen that in the three sprayed plots in the grove at Orlando practically no melanose was found, and the slight trace which is reported may possibly have been spray injury rather than melanose infection. The plot receiving monthly applications was about as well protected as those receiving weekly sprayings, whereas the unsprayed part of the grove had practically 9 out of every 10 fruits infected with melanose and for the most part the degree of injury could be classed as severe.



TABLE 7.—*Spraying experiments with 3-4-50 Bordeaux-oil emulsion for melanose control on orange and grapefruit groves at Orlando and Winter Park, Fla., in 1920*

Location of grove	Number of applications	Date applied	Kind of fruit	Melanose infection	
				Absent	Infected
Orlando.....	18 weekly.....	Apr. 1 to Aug. 4.....	Oranges.....	<i>Per cent</i> 99.4	<i>Per cent</i> 0.6
Do.....	9 biweekly.....	Apr. 1 to July 22.....	do.....	99.6	.4
Do.....	5 monthly.....	do.....	do.....	98.5	1.5
Do.....	Check (unsprayed).....	do.....	do.....	6.95	93.05
Winter Park.....	1.....	May 5 to 8.....	Grapefruit.....	91.13	8.87
Do.....	Check (unsprayed).....	do.....	do.....	32.43	67.57
Do.....	1.....	May 10 to 12.....	Oranges.....	92.17	7.83
Do.....	Check (unsprayed).....	do.....	do.....	5.6	94.4

The grove at Winter Park, 4 miles from Orlando, was sprayed but once for the control of this disease. This application was made during the first part of May before much infection had taken place, although there had been considerable rain up to that time. From

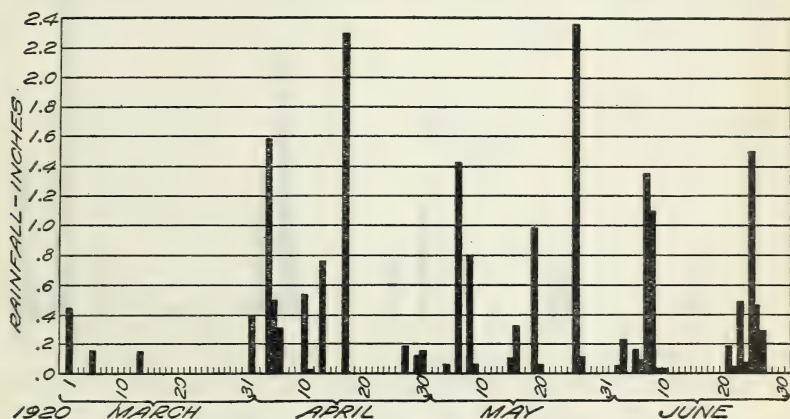


FIG. 5.—Rainfall (in inches) at Orlando, Fla., during March, April, May, and June, 1920

the results of this spraying test, together with the results obtained by the commercial growers who made tests, it seems that a single application of Bordeaux-oil emulsion properly timed should give good control of melanose.

So far as is known this is the first instance where melanose has been successfully controlled by a single spray application.

The results of these tests were made known to the various State workers and horticultural advisers for use during 1921, and rather large numbers of commercial properties were sprayed that year, the material and time of application being based on the results of the 1920 tests given above.

*Rainfall during the spring months of 1920.*—A graphic record of the daily rainfall at Orlando during the melanose season of 1920 is shown in Figure 5. It is obvious that March of that year at Orlando was very dry. No rain fell there during the latter half of

the month, while in April there was an excessive quantity of rainfall to the extent of 4.7 inches above normal, but there were only a few rainy days. This resulted in very little infection before May 5, and from then on several rains fell and much more infection occurred.

#### SPRAYING EXPERIMENTS IN 1921

In 1921 the spraying experiments were conducted in the same seedling orange grove at Orlando which had been used the preceding year. Thirty-eight plots were sprayed, each receiving from one to three applications of fungicide. The following spray materials were used: Lime-sulphur solution, dry lime-sulphur, barium tetra-sulphide, a commercial form of self-boiled lime-sulphur, Pritchard and Clark's (17) copper soap, 3-3-50 Bordeaux mixture plus 1 per cent oil as emulsion, 1-1-50 Bordeaux mixture plus 1 per cent oil, and a commercial Bordeaux powder in proportions to give 3-3-50 Bordeaux mixture to which 1 per cent oil as emulsion was added.

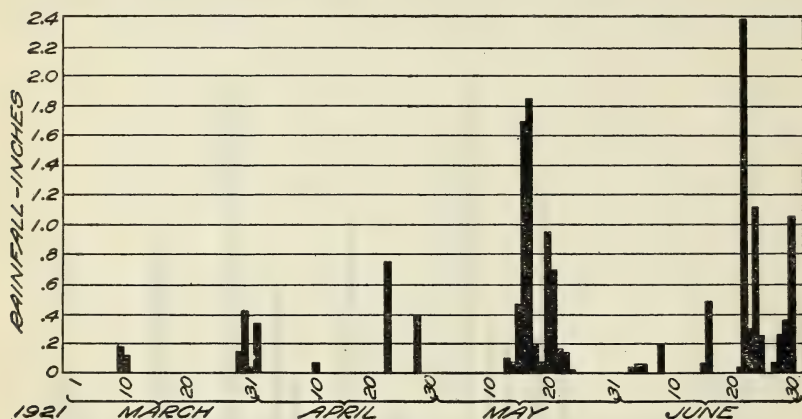


FIG. 6.—Rainfall (in inches) at Orlando, Fla., during March, April, May, and June, 1921

The list of spray materials, dates of application, and results obtained are given in Table 8.

From Table 8 it is obvious that sulphur sprays as a class are practically worthless for melanose control, and that copper soap and the weaker strengths of Bordeaux mixture are only partially effective. Stronger Bordeaux mixture applied about the first of May gave very good control of melanose, but applications made after the middle of that month were decidedly less effective, and those in June had little or no effect on the disease. The added protection resulting from the second and third applications is not sufficient to justify more than one application.

*Rainfall during the spring months of 1921.*—In order to aid in determining the effect rainfall may have had on the efficacy of the sprays, the precipitation record at Orlando during the melanose season of 1921 is shown in Figure 6. It is seen from this figure that very little rain fell during March and April, and May was dry until about the 13th of the month, when rains set in and lasted for 12



consecutive days. No appreciable melanose infection had taken place when the first application was made; however, considerable infection had developed by the time the second application was made, and probably all of the infection had taken place when the third application was put on.

TABLE 8.—*Spraying experiments for melanose control with various materials on orange and grapefruit trees at Orlando, Fla., in 1921*

Plot No.	Materials used	Number of applications	Proportions	Dates applied	Percentage of melanose infections	Degree of infection
1	Lime-sulphur solution.	1	1-40.....	Apr. 28.....	100	Very severe on all grapefruit trees and on larger orange trees.
2	do.....	2	do.....	Apr. 28, May 23..	100	Do.
3	do.....	3	do.....	Apr. 28, May 17, June 7.	100	Do.
4	do.....	1	do.....	May 17.....	100	Do.
5	do.....	1	do.....	June 7.....	100	Do.
6	Dry lime-sulphur.	1	4-40.....	Apr. 28.....	100	Do.
7	do.....	2	do.....	Apr. 28, May 24..	100	Do.
8	do.....	3	do.....	Apr. 28, May 17, June 7.	100	Do.
9	do.....	1	do.....	May 28.....	100	Do.
10	do.....	1	do.....	June 7.....	100	Do.
11	Barium tetrasulphide.	1	6-40.....	Apr. 28.....	100	Do.
12	do.....	2	do.....	Apr. 28, May 24..	100	Do.
13	do.....	3	do.....	Apr. 28, May 17, June 7.	100	Do.
14	do.....	1	do.....	May 28.....	100	Do.
15	do.....	1	do.....	June 7-8.....	100	Do.
16	Commercial self-boiled lime sulphur.	1	4-5-40.....	Apr. 29.....	99	Do.
17	do.....	2	do.....	Apr. 29, May 24..	99	Do.
18	do.....	3	do.....	Apr. 29, May 17, June 8.	100	Do.
19	do.....	1	do.....	May 28.....	100	Do.
20	do.....	1	do.....	June 8.....	100	Do.
21	Copper soap	1	½-0-3-50.....	Apr. 29.....	60	Slight.
22	do.....	2	do.....	Apr. 29, May 20..	68	Do.
23	do.....	3	do.....	Apr. 29, May 18, June 8-9.	82	Do.
24	do.....	1	do.....	May 20.....	100	Medium.
25	do.....	1	do.....	June 8.....	100	Severe.
26	Bordeaux oil	1	3-3-50+1 per cent oil.	Apr. 29.....	5	Very slight.
27	do.....	2	do.....	Apr. 29, May 30..	3	Do.
28	do.....	3	do.....	Apr. 29, May 19, June 9.	0	
29	do.....	1	do.....	May 19.....	66	Medium.
30	do.....	1	do.....	June 9.....	82	Severe.
31	do.....	1	1-1-50+1 per cent oil.	Apr. 30.....	50	Slight.
32	do.....	2	do.....	Apr. 30, May 23..	78	Medium.
33	do.....	3	do.....	Apr. 30, May 19, June 9.	0	Do.
34	do.....	1	do.....	May 23.....	100	Very severe.
35	do.....	1	do.....	June 9.....	100	Do.
36	Commercial Bordeaux powder.	1	3-3-50+1 per cent oil.	May 23.....	100	Do.
37	do.....	2	do.....	May 23, June 8..	100	Do. ✓
38	do.....	1	do.....	June 8.....	100	Do.

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*Tests for copper on sprayed leaves.*—In order to determine the persistence of Bordeaux mixture spray residue, quantitative tests for the presence of copper were made from leaves sprayed with two strengths of Bordeaux mixture plus oil emulsion, viz. 3-3-50 plus 1 per cent oil as compared with 1-1-50 plus 1 per cent oil. The results are shown in Figure 7.

It will be seen from Figure 7 that very much more copper can be recovered from leaves of trees sprayed with 3-3-50 Bordeaux-oil emulsion than with a 1-1-50 Bordeaux-oil emulsion. If it is assumed that 15 milligrams of copper per 100 grams of fresh leaves is necessary to afford protection against melanose, it is obvious that the 1-1-50 Bordeaux-oil does not meet the requirements for any great length of time, whereas a single application of Bordeaux-oil emulsion in late April carries about enough copper to afford protection throughout the entire period of infection. It is also shown that three

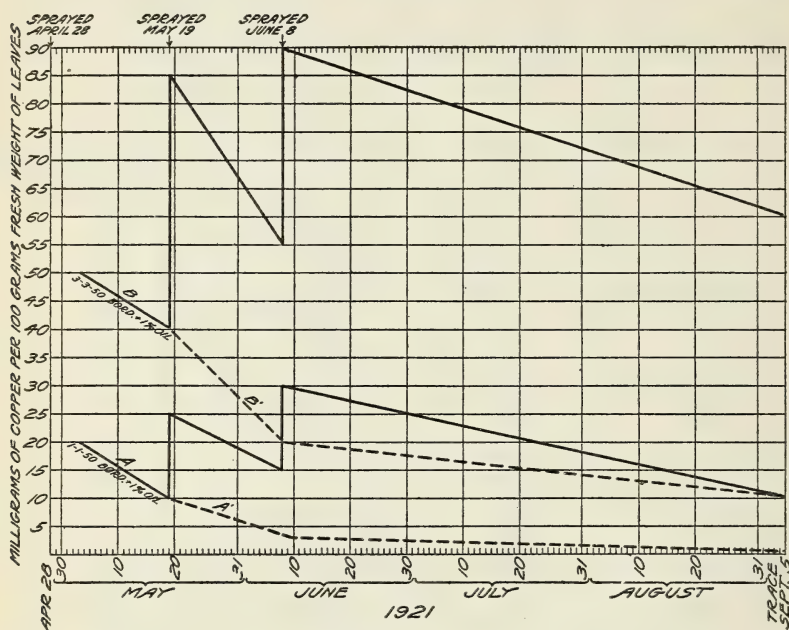


FIG. 7.—Quantities of copper remaining in spray residue on citrus leaves during a 4-month period. Four sprayed plots were sampled: A, sprayed three times with 1-1-50 Bordeaux plus 1 per cent oil; A', sprayed once with the same material; B, sprayed three times with 3-3-50 Bordeaux plus 1 per cent oil; B', sprayed once with the same material. The first spraying was on April 28. The first sampling of leaves was on May 3.

applications of a 1-1-50 Bordeaux-oil emulsion weathers down during the rainy season about to the point of a single application of 3-3-50 strength.

*Other experiments in 1921.*—The Florida Agricultural Experiment Station in 1921 conducted spraying experiments based largely on the results obtained by this office during 1920 and obtained very satisfactory control from single applications made before the heavy infection of May took place. These tests, together with experiments in 1922, were reported by Burger, DeBusk, and Briggs (2).

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Spraying experiments were repeated in 1922 in the same grove that was used the preceding year. The first applications were made between April 10 and 14 and the late applications as late as June 10.

#### SPRAYING EXPERIMENTS IN 1922



TABLE 9.—*Spraying experiments for melanose control with various materials on orange and grapefruit trees at Orlando, Fla., in 1922*

Plot No.	Materials used	Number of applications	Proportions	Date applied	Melanose infections					
					Absent	Very slight	Slight	Medium	Severe	Very severe
					Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	Lime-sulphur solution.	3	1-40-----	Apr. 13 and 14, 28, May 23.	0	21	26	32	17	4
2	Copper soap-----	1	½-0-3-50-----	Apr. 13-----	0	5	11	19	59	6
3	do-----	2	do-----	Apr. 13, 28-----	1	7	22	20	47	3
4	do-----	3	do-----	Apr. 13, 28, May 23.	46	17	17	14	4	2
5	Bordeaux-oil emulsion.	1	1-1-50+1 per cent oil.	Apr. 11-----	6	15	14	27	35	3
6	do-----	2	do-----	Apr. 11, 29-----	13	19	30	24	13	1
7	do-----	3	do-----	Apr. 11, 29, May 23	88	8	3	1	0	0
8	do-----	1	2-2-50+1 per cent oil.	Apr. 11-----	40	27	20	9	3	1
9	do-----	2	do-----	Apr. 11, 29-----	46	26	19	8	1	0
10	do-----	3	do-----	Apr. 11, 29, May 22	89	6	5	0	0	0
11	Neutral Bordeaux-oil emulsion.	1	3-x-50+1 per cent oil.	Apr. 13-----	90	5	4	1	0	0
12	do-----	2	do-----	Apr. 13, 28-----	99	1	0	0	0	0
13	do-----	3	do-----	Apr. 13, 28, May 22	95	3	2	0	0	0
14	Commercial Bordeaux-oil emulsion.	2	Approximately 2-x-50+¾ per cent oil.	Apr. 13, 29-----	96	2	2	0	0	0
15	do-----	3	do-----	Apr. 12, 29, May 22	98	2	0	0	0	0
16	Improperly prepared Bordeaux-oil emulsion.	2	3-3-50+1 per cent oil.	Apr. 12, 28-----	94	4	2	0	0	0
17	do-----	3	do-----	Apr. 12, 28-29, May 23.	97	2	1	0	0	0
18	Bordeaux-oil emulsion.	1	do-----	Apr. 10-----	79	8	10	2	1	0
19	do-----	2	do-----	Apr. 10, May 1-----	97	1	1	1	0	0
20	do-----	3	do-----	Apr. 10, May 1, 22.	97	1	1	1	0	0
21	do-----	3	do-----	May 1, 22, June 9.	99	1	0	0	0	0
22	do-----	3	do-----	Apr. 10-11, 25, May 10.	95	2	2	1	0	0
23	do-----	3	do-----	Apr. 25, May 10, 25	95	2	2	1	0	0
24	do-----	3	do-----	May 10, 25, June 9.	92	4	3	1	0	0
25	do-----	2	do-----	May 25, June 9-10.	93	4	2	1	0	0
26	do-----	2	do-----	Apr. 12, May 10-----	94	3	2	1	0	0
27	do-----	3	do-----	Apr. 12, May 10, June 10.	98	1	1	0	0	0
28	do-----	2	do-----	Apr. 20, May 20-22	90	4	5	1	0	0
29	do-----	2	do-----	May 1, June 1-----	90	5	3	2	0	0
30	do-----	2	do-----	May 10-11, June 10	81	6	9	4	0	0
31	do-----	1	do-----	Apr. 20-----	38	32	24	6	0	0
32	do-----	1	do-----	May 1-----	62	24	12	2	0	0
33	do-----	1	do-----	May 11; no fruit-----						
34	do-----	1	do-----	May 20-----	88	6	4	2	0	0
35	do-----	1	do-----	June 1-----	15	23	25	22	12	3
36	do-----	1	do-----	June 10-----	0	1	12	29	56	2
37	do-----	8	do-----	Apr. 12, May 11, June 10, July 10, Aug. 12, Sept. 13, Oct. 13, Nov. 10.	91	6	3	0	0	0
Check	Average of 12 no-treatment plots.				0	3	27	31	23	16

In view of the failure of sulphur sprays as a class to control melanose in 1921, it was considered unnecessary to use again a wide range of these materials. The following materials were tested: Lime-sulphur solution, copper soap, various strengths of alkaline Bordeaux mixture plus oil emulsion, improperly prepared <sup>6</sup> Bordeaux mixture

<sup>6</sup> Bordeaux mixture made by pouring concentrated milk of lime into concentrated blue-stone solution.

plus oil emulsion, "neutral"<sup>7</sup> Bordeaux plus oil emulsion, and a commercial preparation of Bordeaux mixture and oil emulsion.

A complete list of spray materials, dates of application, and results obtained are given in Table 9.

Again it will be seen that lime-sulphur solution failed to give any appreciable control of melanose. One and two applications of copper soap afforded a slight protection, and three applications gave a considerably greater protection but not in proportion to the cost involved. The same applies to 1-1-50 Bordeaux-oil emulsion and to a less extent to 2-2-50 Bordeaux and oil. Two and three applications of commercial Bordeaux-oil emulsion gave excellent results, likewise one or two applications of standard 3-3-50 Bordeaux and oil gave good commercial control of the disease whenever the spraying was done in advance of the May rains.

*Rainfall during the spring months of 1922.*—Figure 8 shows the daily precipitation at Orlando during the melanose season of 1922. This figure shows that the spring of 1922 was unusually dry. Many

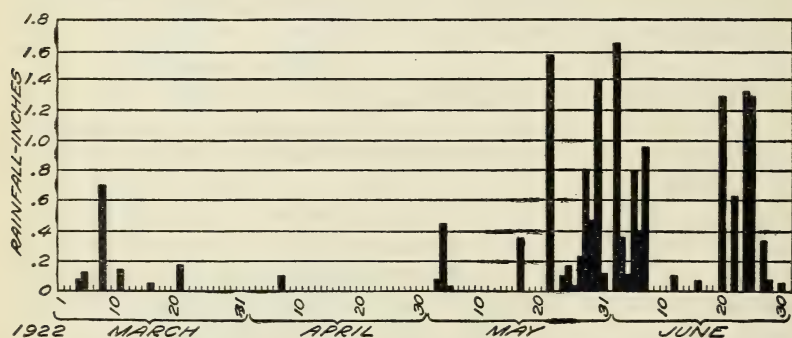


FIG. 8.—Rainfall (in inches) at Orlando, Fla., during March, April, May, and June, 1922

twigs died from drought effects and later became a source of melanose infection. The drought was broken early in May, when some infection developed. After the middle of the month rains occurred frequently and the resulting infection was abundant.

*Copper tests.*—Again copper tests were made from leaves sprayed with various copper materials, data from which are shown in Figure 9.

These findings are in accord with those of the preceding year—namely, that a much greater quantity of copper is found on trees sprayed with 3-3-50 Bordeaux and oil than those receiving sprays carrying smaller quantities of copper. It seems probable that a single application of 3-3-50 Bordeaux and oil at almost any time in April would afford a fair commercial control of the disease, perhaps better than three applications of copper soap or two applications of a 1-1-50 Bordeaux mixture. The 2-x-50 commercial Bordeaux and oil does not seem to persist on orange leaves quite as well as home-made 2-2-50 Bordeaux and oil.

<sup>7</sup> Bordeaux mixture made by pouring slowly just enough concentrated milk of lime into dilute bluestone solution in agitation to precipitate all of the soluble copper as indicated by the potassium-ferrocyanide test. This neutral Bordeaux mixture is indicated by a formula such as 3-x-50.



## SPRAYING EXPERIMENTS IN 1923

Spraying experiments were continued in 1923 at Orlando on seedling orange and grapefruit trees and at two other points in the State on grapefruit. Thirty-five plots were sprayed at Orlando with

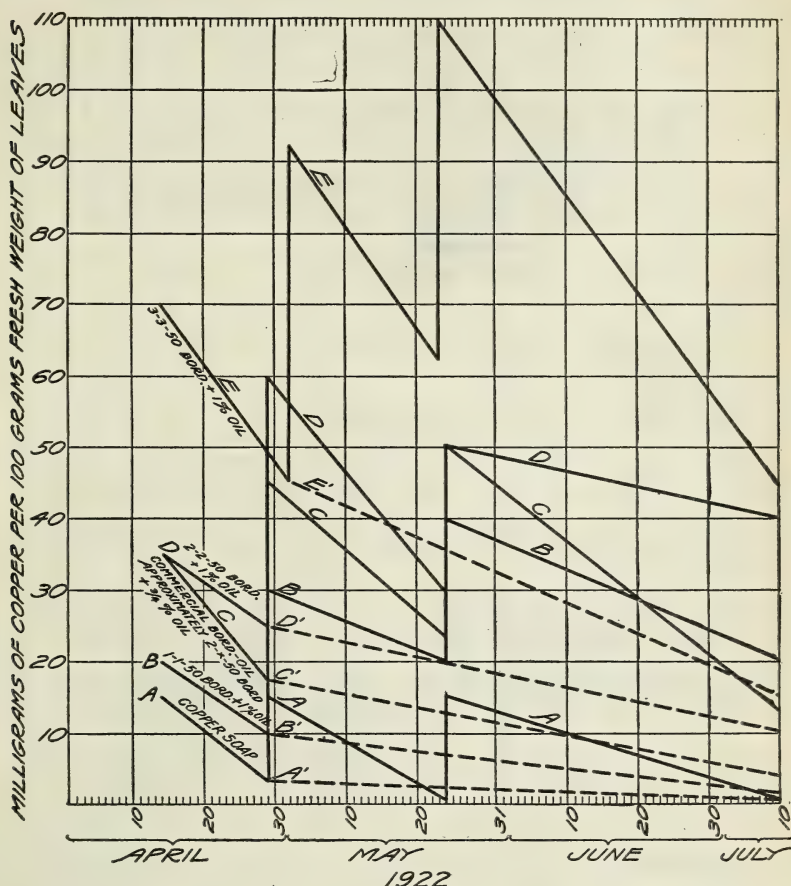


FIG. 9.—Quantities of copper remaining in spray residue on citrus leaves during a 3½-month period. Five spray mixtures were used, and these were applied three times to certain plots (A, B, C, D, E), and once to other plots (A', B', C', D', E'). The first application was on April 14

various copper compounds or with lime-sulphur solution for the control of melanose.

In former years the only oil emulsions used were prepared with soap, but this season the soap-oil emulsions were used in comparison with kaolin-oil emulsions (27). Since no appreciable difference in effects could be noted between the two types of emulsion in Bordeaux mixture, a discussion of their respective merits is not given.

The first application was made as early as April 3 and the last was made on June 15, the plots receiving from one to three applications respectively. The materials used, dates and number of applications, together with the degree of melanose control obtained on the sprayed plots, are given in Table 10.

TABLE 10.—*Results of spraying experiments for melanose control with various materials on orange and grapefruit trees at Orlando, Fla., in 1923*

Materials used	Number of applications	Proportions	Date applied	Melanose infection					
				Absent	Very slight	Slight	Medium	Severe	Very severe
Bordeaux-oil emulsion...	3	3-4-50+1 per cent oil.	May 5, 15, June 5...	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Do.....	3	do.....	Apr. 24, May 7, 23...	2	37	23	28	9	1
Do.....	3	do.....	Apr. 5, 24, May 15...	54	44	2	0	0	0
Improperly prepared Bordeaux mixture.	1	do.....	May 2.....	64	32	4	0	0	0
'Neutral' Bordeaux mixture.	1	3-x-50+1 per cent oil.	do.....	50	42	6	1	1	0
Commercial Bordeaux paste. <sup>1</sup>	1	3-3-50+1 per cent oil.	do.....	65	29	6	0	0	0
Commercial Bordeaux-oil emulsion. <sup>2</sup>	1	do.....	do.....	40	50	9	1	0	0
Do. <sup>3</sup> .....	1	do.....	do.....	3	64	32	1	0	0
Do.....	1	2-2-50+¾ per cent oil.	May 4.....	0	30	44	20	6	0
Do.....	1	3-3-50+1 per cent oil.	do.....	0	51	41	7	1	0
Ammoniacal solution of copper carbonate.	1	5-3-50.....	do.....	0	40	29	24	7	0
Bordeaux-oil emulsion...	1	2-2-50+1 per cent oil.	May 2.....	35	59	5	1	0	0
Do.....	1	5-5-50+1 per cent oil.	do.....	34	63	3	0	0	0
Do.....	1	4-4-50+1 per cent oil.	do.....	56	33	10	1	0	0
Do.....	2	3-4-50+1 per cent oil.	Apr. 16, May 23.....	53	43	4	0	0	0
Do.....	2	do.....	Apr. 24, May 23.....	41	54	5	0	0	0
Do.....	2	do.....	May 5, 23.....	6	28	32	22	11	1
Lime-sulphur solution...	3	1-40.....	Apr. 25, May 7, 25.....	14	58	24	3	1	0
Do.....	2	do.....	May 4, 25.....	23	44	22	10	1	0
Bordeaux-oil emulsion...	3	3-4-50+1 per cent oil.	Apr. 25, May 15, June 6.....	65	35	0	0	0	0
Do.....	2	do.....	Apr. 25, May 15.....	81	18	1	0	0	0
Do.....	2	do.....	Apr. 16, May 5.....	41	26	32	1	0	0
Do.....	2	do.....	Apr. 11, June 6.....	25	49	23	3	0	0
Do.....	2	do.....	Apr. 11, May 24.....	60	37	3	0	0	0
Do.....	2	do.....	Apr. 11, May 15.....	64	33	3	0	0	0
Do.....	2	do.....	Apr. 11, May 7.....	67	33	0	0	0	0
Do.....	3	do.....	Apr. 11, 25, May 15.....	79	21	0	0	0	0
Do.....	2	do.....	Apr. 11, 25.....	67	32	1	0	0	0
Do.....	1	do.....	June 15.....	12	41	33	13	1	0
Do.....	1	do.....	June 6.....	47	43	8	1	1	0
Do.....	1	do.....	May 24.....	52	35	10	3	0	0
Do.....	1	do.....	May 15.....	12	64	20	3	1	0
Do.....	1	do.....	May 4.....	33	48	15	3	1	0
Do.....	1	do.....	Apr. 25.....	47	44	9	0	0	0
Do.....	1	do.....	Apr. 16.....	3	33	36	24	4	0
Do.....	1	do.....	Apr. 11.....	0	36	40	18	6	0
Do.....	1	do.....	Apr. 3.....	10	36	32	19	3	0
Average of 12 check plots (no treatment).				0	1	26	25	34	14

<sup>1</sup> A commercial Bordeaux paste to which oil emulsion was added in the spray tank.<sup>2</sup> A ready-mixed commercial Bordeaux-oil emulsion, type A.<sup>3</sup> A ready-mixed commercial Bordeaux-oil emulsion, type B.

It is evident that the control was not as perfect as in former years. This is doubtless due to a slight but general infection that occurred in late March.

The plots sprayed with a standard 3-3-50 Bordeaux plus 1 per cent oil as emulsion or its equivalent before the middle of April were not as well protected as those sprayed just before the May rains set in, while those sprayed for the first time well after the rains set in were in general not so satisfactorily protected. From these tests there is again evidence that if a single spray application is made before the May rains set in, melanose infection will be largely eliminated; that the commercial Bordeaux mixtures plus oil gave satisfactory results;



and that sulphur and weaker copper sprays are not satisfactory against this disease. There seems to be no commercial advantage in using more than one application of 3-3-50 Bordeaux plus oil emulsion if this is properly timed. It is also evident that an application made several weeks in advance of the main outbreak is not as effective as one put on just ahead of the principal infection period.

*Rainfall during the spring months of 1923.*—The rainfall at Orlando, Fla., during the spring months of 1923 is shown in Figure 10 as an aid in interpreting the results obtained in the spraying experiments. Figure 10 shows that March was rainy, that April was not especially dry, and that the May rains set in on the 3d of that month.

A slight amount of melanose showed up plainly on the new leaves and fruit in late March, but it was not until the rainy period of early May that severe infection occurred. After the middle of May the

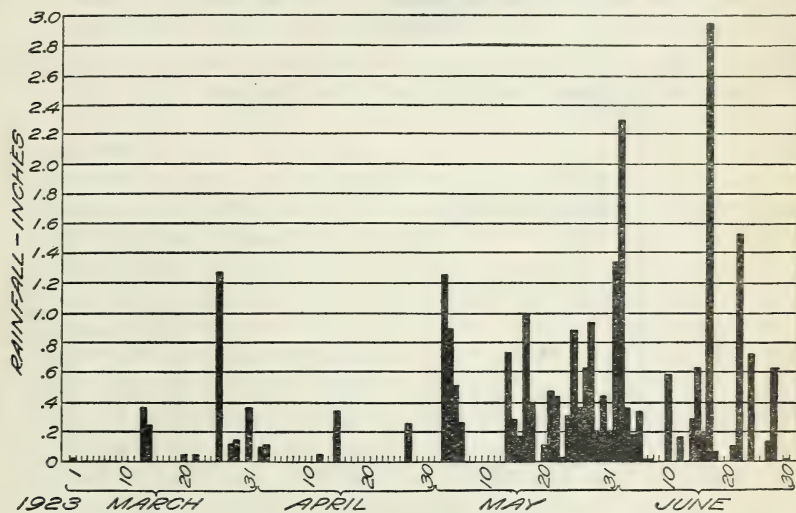


FIG. 10.—Rainfall (in inches) at Orlando, Fla., during March, April, May, and June, 1923

weather was especially conducive to infection, but by that time most of the fruit had grown too large and tough to become further infected. This resulted in a slight March infection on practically all fruit, but owing to the fact that the fruit had become unusually large by the middle of May a noticeably small proportion was blemished very severely by this disease.

A grapefruit grove near Leesburg in Lake County, Fla., and another near Brownville in De Soto County were sprayed experimentally during February and March for scab control, and counts were also made for the effect of these early sprays on the prevention of melanose. The first application was made just as the spring leaf buds were swelling, the second toward the end of the blooming period, and the third, when applied, three weeks later. In general, melanose infection was about as severe on the plots receiving sulphur sprays as on the unsprayed plots, but the plots receiving one, two, and three applications of Bordeaux mixture during the early growing period were fairly well protected from the disease. From these experi-

mental data, together with the results obtained in former years on plots sprayed for citrus scab control, as well as in commercial groves in 1923, it is evident that melanose can be reduced decidedly by applications of Bordeaux mixture during the scab-spraying season. But, generally speaking, much better results are obtained if the application is delayed until the latter part of April or the first of May, provided the weather during April is not extremely wet.

## SPRAYING EXPERIMENTS IN 1924

In 1924 spraying experiments were again conducted at Orlando in a mixed grapefruit and seedling-orange grove in which 31 sets of duplicated plots were sprayed with copper or sulphur sprays, but for the most part Bordeaux-oil emulsion was used. Spraying operations were begun March 17, before many blooms had opened, and they were discontinued after June 28. Plots received from one to three applications at different times, as is shown in Table 11.

TABLE 11.—Results of spraying experiments for melanose control with various materials on orange and grapefruit trees at Orlando, Fla., in 1924

Materials used	Number of applications	Proportions	Date applied	Melanose infection					
				Absent	Very slight	Slight	Medium	Severe	Very severe
				Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Bordeaux oil emulsion	1	3-3-50+1½ per cent oil.	Mar. 17	19	32	29	13	5	2
Do	1	do	Mar. 27	35	31	25	8	1	0
Do	1	do	Apr. 7	63	19	10	6	2	0
Do	1	do	Apr. 17	66	17	7	8	2	0
Do	1	3-3-50+1 per cent oil.	Apr. 26	84	11	5	0	0	0
Do	1	do	May 7	70	15	11	4	0	0
Do	1	do	May 16	74	17	6	3	0	0
Do	1	do	May 26	70	19	9	2	0	0
Do	1	do	June 6	60	23	14	3	0	0
Do	1	3-3-50+1½ per cent oil.	June 16	0	20	32	37	9	2
Do	1	do	June 28	0	17	30	31	18	4
Do	2	3-3-50+1½ and 1 per cent oil.	Apr. 3, May 23	64	21	11	4	0	0
Do	2	do	Apr. 3, May 1	81	12	6	1	0	0
Do	2	do	Apr. 7, May 24	78	14	7	1	0	0
Do	2	do	Apr. 7, May 1	72	16	7	4	1	0
Do	3	3-3-50+1½, 1, and 1 per cent oil	Apr. 8, May 1, 22	86	6	5	2	1	0
Do	3	do	Apr. 17, 30, May 10	83	12	3	2	0	0
Do	2	3-3-50+1½ and 1 per cent oil.	Apr. 21, 28	82	13	4	1	0	0
Do	2	do	Apr. 21, May 10	80	13	6	1	0	0
Do	2	do	Apr. 21, May 20	79	13	7	1	0	0
Do	2	3-3-50+1 per cent oil.	Apr. 26, May 10	77	16	6	1	0	0
Do	2	do	Apr. 28, May 20	83	11	5	1	0	0
Do	2	do	Apr. 25, May 26	82	13	4	1	0	0
Do	2	do	May 7, 21	77	14	7	1	1	0
Colloidal sulphur	2	5-50	Apr. 24, May 17	25	30	27	16	2	0
Lime-sulphur solution	2	1-40	Apr. 23, May 17	15	36	25	19	3	2
Lime-sulphur sludge	2	do	Apr. 24, May 16	29	33	28	8	2	0
Neutral copper acetate	2	1-50	Apr. 23, May 19	31	35	29	4	1	0
Do	1	do	Apr. 23	29	31	33	5	2	0
Bordeaux-oil emulsion	1	6-6-50+1 per cent oil.	Apr. 22	65	16	10	7	2	0
Bordeaux mixture	1	3-3-50	Apr. 19	42	22	25	8	3	0
Average of 13 check plots having no treatment.				2	13	24	36	17	8



The crop of *Phomopsis* spores was unusually large in March and April, and everything pointed to a heavy fruit infection with the onset of the rains. This was particularly true in the grove where the experimental spraying was being conducted.

The spray materials used, dates and numbers of applications, together with the results obtained from this experimental spraying for melanose control in 1924 are given in Table 11.

Again, it is shown that a single application of Bordeaux-oil emulsion applied before the period of heavy melanose infection gives a good commercial control of the disease. Bordeaux-oil emulsion applied March 17, even before many blooms opened, had a marked effect upon melanose infections. Progressively later applications up to the onset of long rainy periods increasingly gave better results, but wherever the sprays were applied well after the June rains had set in the control was not satisfactory.

Two and three applications of Bordeaux-oil emulsion gave somewhat better results than single applications, but the difference was

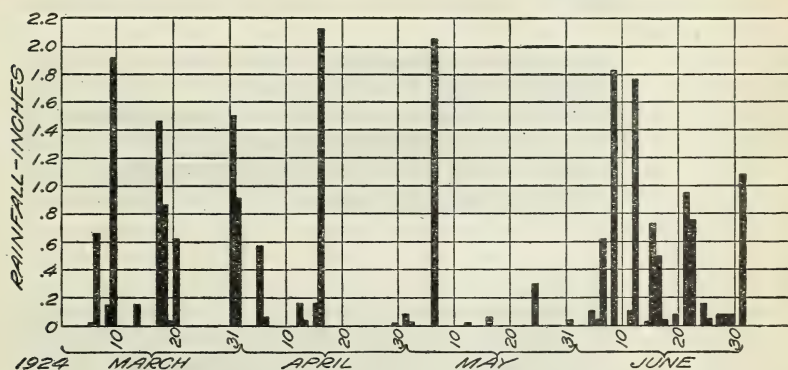


FIG. 11.—Rainfall (in inches) at Orlando, Fla., during March, April, May, and June, 1924

not sufficiently great to warrant the expense of more than a single application properly timed.

Neutral copper acetate and the sulphur compounds are not nearly so effective against melanose as standard Bordeaux-oil emulsion.

*Rainfall during the spring months of 1924.*—The weather in the neighborhood of Orlando in the spring of 1924 was very unusual. Because of the cold, wet spring, growth was slow in getting started, and the blooming period hung on much later than normal. A heavy melanose infection on leaves occurred during March, but because of the delayed bloom there was very little fruit then exposed to infection. April was fairly dry and May likewise was not especially wet.

Slight fruit infection developed in late April and early May, and somewhat more occurred during the latter part of that month, but it was not until June that heavy fruit infection occurred—a month later than usual.

The rainfall at Orlando, Fla., during the spring months of 1924 is shown in Figure 11.

## SUMMARY OF EXPERIMENTAL PLOTS SPRAYED ONCE

Figure 12 shows in diagrammatic form the relative effectiveness during three seasons of single applications of Bordeaux-oil emulsion at 10-day intervals over a period of about two months, beginning about April 1. These are once-sprayed orange plots from the experiments reported in the foregoing pages.

It will be seen that effective control was obtained in 1922 from April 10 to May 20, in 1923 from April 25 to June 6, and in 1924

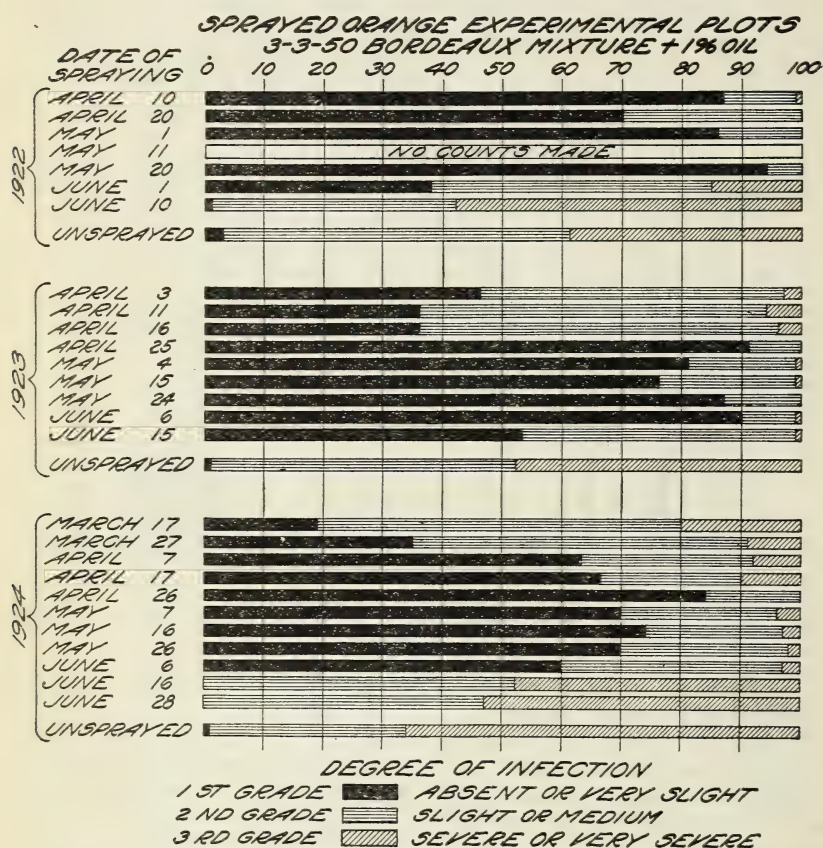


FIG. 12.—Percentages of first, second, and third grade oranges, with respect to melanose blemish, from plots sprayed once with 3-3-50 Bordeaux mixture plus 1 per cent oil, the application being made to the various plots on successive dates, to determine the best time to spray for melanose control. Results for three years are shown

from April 7 to June 6. It further appears that on the average nothing was gained by spraying before April 15 and that in some years there was an advantage in waiting until about April 25 to make the application for melanose, the delay favoring the effective persistence of the spray coating during the whole of the five or six weeks' danger period following. For the three years in question under the conditions prevailing in this test grove there would have been no serious loss in effectiveness from delaying the application



until May 15. But the greater the delay the greater the danger of being caught unprotected, and it is certainly wise to get the work done promptly in the first part of what seems to be the best period, which would mean between April 25 and May 5.

#### TESTS OF SUMMER AND FALL SPRAYING FOR MELANOSE CONTROL

In 1920 spraying experiments were conducted during September, October, and November with Bordeaux-oil emulsion on seedling orange trees. The same material was used from June to November of 1921. In 1922 and again in 1923 similar spraying experiments were conducted during the summer months only. This summer and fall spraying was done too late to prevent or even reduce melanose infection on fruit then on the trees, and it also failed to check melanose the following year. From these rather extensive spraying experiments it is evident that spraying in the summer and fall can not be expected to reduce melanose outbreaks the following year.

#### COMMERCIAL SPRAYING

*Commercial spraying in 1920.*—Prior to 1920 no commercial spraying was done in Florida for melanose control, but that year several growers sprayed their groves for the prevention of this blemish. The dates of applications and spray materials were suggested by this office. For the most part 3-3-50 Bordeaux plus 1 per cent oil as emulsion was used. Among the several groves sprayed that year for the control of the disease was a large property in Marion County, another in Polk County, and a third in Pinellas County. In all of the groves melanose had been a major factor for many years, as there was an abundance of deadwood in them. Parts of these groves were sprayed from one to four times, the first application being made in late April.

Although actual counts were not made in any of these groves to determine the exact control, close observation led the writers to believe that in general the melanose control was very satisfactory in these three properties, about as good as in the groves sprayed experimentally at Winter Park and Orlando; but injurious effects developed in the form of excessive scale infestation owing to the omission of insecticides during the summer months.

*Commercial spraying in 1921.*—By 1921 the results of the successful spraying experiments and commercial tests carried on in 1920 whereby melanose was successfully controlled in several parts of Florida by a single application of Bordeaux-oil emulsion had become generally known. Perhaps 20 or more groves were sprayed in 1921 at such times as to have the job completed by about May 1. In every instance the disease was reduced markedly and in most cases the control was almost perfect. On an average fully 90 per cent of the sprayed fruit matured commercially free of the disease. Most of these groves were sprayed in summer for scale control, but those few which were not so sprayed thoroughly with oil emulsion had a heavy scale infection by fall.

*Commercial spraying in 1922.*—By 1922 growers generally had become much interested in spraying for melanose because of the success of the preliminary spraying trials and because the market was show-

ing a decided preference for fruit free of blemishes. That year a large number of groves were sprayed for the prevention of this disease. For comparison with results obtained in experimental spraying, a careful check was made on a number of these groves scattered well over the entire citrus belt. The counts were made in

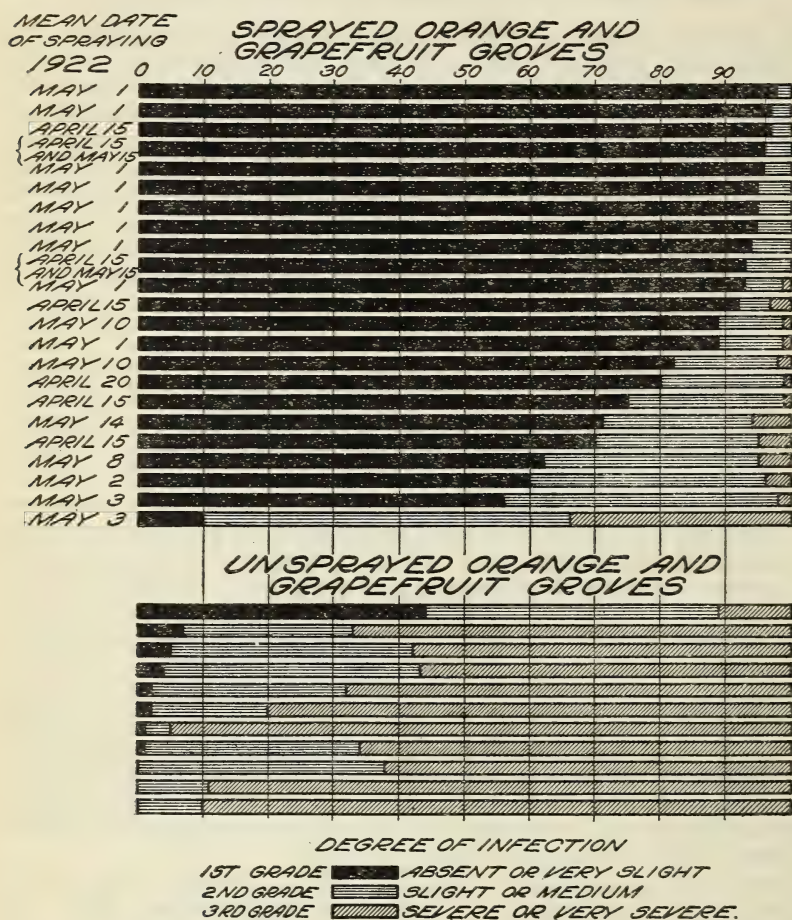


FIG. 13.—Percentages of first, second, and third grade fruit, with respect to melanose blemish, from groves sprayed by the owners in 1922 with Bordeaux-oil emulsion. Grading of fruit in similar groves that were not sprayed is given for comparison

the same manner and by the same persons who made final counts on the experimental plots. In order to present the data from these counts in simple graphic form, fruits free of melanose blemishes and those only slightly affected were arbitrarily combined into the first grade; fruits slightly and moderately affected were placed in the second grade; and those severely and very severely affected were placed in the third grade. The results obtained in 23 groves by commercial growers in spraying orange and grapefruit trees for melanose in 1922 are shown in Figure 13.



From these results it is evident that some groves were much better protected than others. The maximum protection obtained was 98 per cent, whereas the minimum protection was only 10 per cent. This variation is to be expected and seems due largely to difference in thoroughness of application, as it is known that the two groves with the least control were not thoroughly sprayed. In some instances, especially on large properties, spraying was not completed until long after heavy infection had taken place. In general, however, melanose control was very satisfactory, and for the most part scale insects did not become abundant during the summer because in nearly all of the groves a special application of oil spray was made in late June. In one or two groves this summer oil spray was omitted, resulting in serious damage from scale insects.

In most instances the unsprayed checks were a part of the sprayed groves, but occasionally near-by unsprayed groves of a like age and general condition were used for checks. Typical scenes in groves sprayed commercially are shown in Plate 10, B, C. The combined results of these tests are given in Table 12.

TABLE 12.—Combined results of commercial spraying for melanose control on orange and grapefruit groves at various points in Florida in 1922

Treatment	Variety	Number of groves	Melanose infection					
			Absent	Very slight	Slight	Medium	Severe	Very severe
			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Sprayed.....	Orange.....	8	64	23	9	3	1	0
Unsprayed.....	do.....	3	1	15	14	27	27	16
Sprayed.....	Grapefruit.....	15	59	21	10	6	3	1
Unsprayed.....	do.....	8	0	2	6	15	26	51
Sprayed.....	Orange and grapefruit.....	23	61	21	10	5	2	1
Unsprayed.....	do.....	11	0	6	9	18	26	41

Again there is considerable evidence to show that the orange is less susceptible to melanose than is the grapefruit, and, as would be expected, melanose control was more complete on the former than on the latter. Comparing the sprayed fruit with the unsprayed, there is a marked reduction in the percentage of blemished fruits as well as a reduction in the degree of injury on the sprayed fruit.

The weather throughout the citrus belt in the spring of 1922 was about normal as regards rainfall during March, but April was very dry, bordering on drought in many sections, and May was very wet and conducive to melanose outbreaks, especially after the 10th or 12th of the month.

*Commercial spraying in 1923.*—In 1923 a still larger number of groves were sprayed commercially with Bordeaux mixture and oil emulsion for the control of melanose, and counts were made in places throughout the citrus belt. Counts were made at points from Jupiter to Hastings along the east coast, at various points throughout the central part of Florida, and from Sutherland to Fort Myers along the west coast. The results of these counts of commercial spraying for melanose control on oranges and on grapefruit in 1923 are shown in Figures 14 and 15.

Like the experimental spraying, the results of these commercial tests are not quite so striking as was the case in 1922. The maximum control on oranges is 100 per cent, and the minimum only 5 per cent, and this minimum control is apparently no better than the best of the unsprayed groves. There is a maximum control of 99 per cent on grapefruit and a minimum control of 0 per cent on the sprayed groves. The latter is not as good as the best of the unsprayed groves.

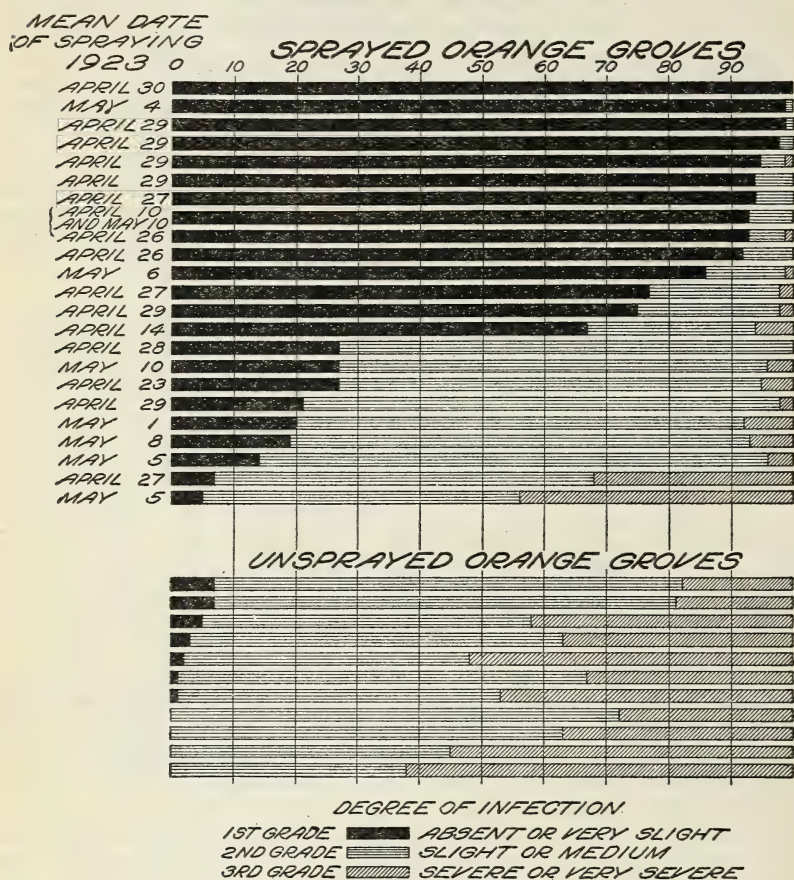


FIG. 14.—Comparison of grading of fruit with respect to melanose blemish in sprayed and unsprayed orange groves in 1923. Bordeaux-oil emulsion was used by the owners

The combined results of the commercial spraying tests are given in Table 13.

Table 13 shows a surprisingly small proportion of fruit entirely free of melanose markings and also a very small proportion showing melanose as occurring in a very high degree. In general, applications of Bordeaux-oil emulsion made for citrus-scab control before April 1 gave fairly good control of melanose, but it was more satisfactory wherever the applications were made just in advance of the onset of the May rains. Applications later than this were much less effective.



Commercial Bordeaux preparations gave satisfactory results, but not as good as the homemade material. This is doubtless due in part at least to the fact that much of the commercial material was used at the rate of a 2-2-50 dilution. Similar-strength homemade Bordeaux mixture is noticeably less effective than the full-strength

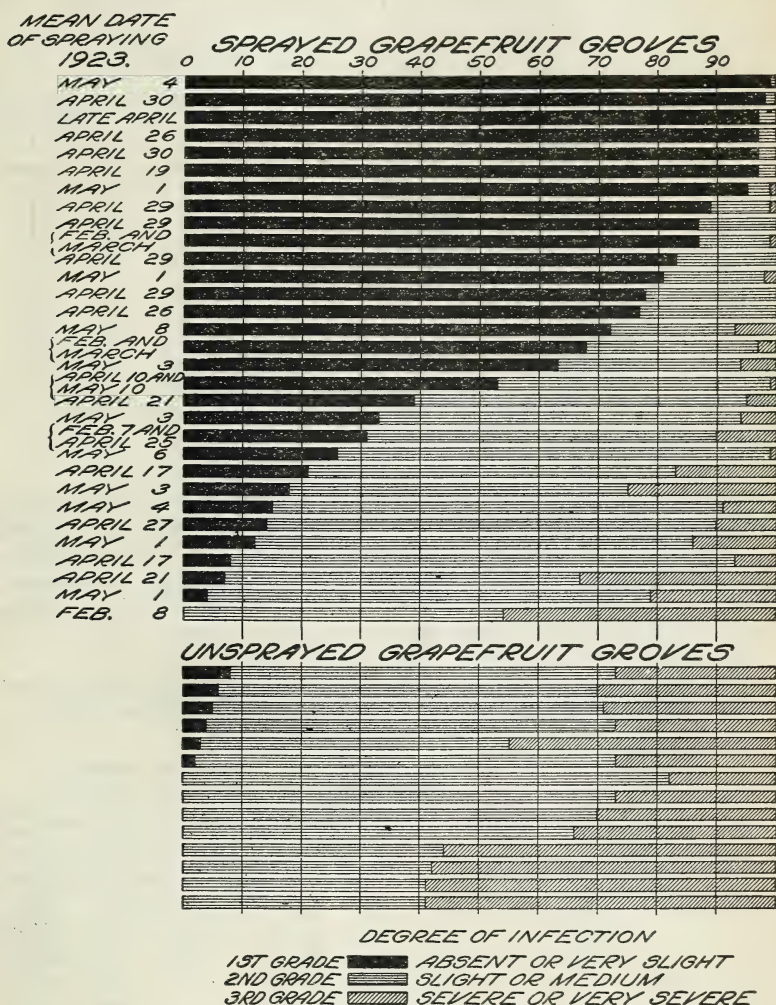


FIG. 15.—Comparison of grading of fruit with respect to melanose blemish in sprayed and unsprayed grapefruit groves in 1923. Bordeaux-oil emulsion was used by the owners

3-3-50 formula. As has been the case in the past, better control was obtained on oranges than on grapefruit.

The weather during the latter half of March and the first week of April was accompanied by occasional general light rains. These rains resulted in a moderate melanose infection throughout the citrus belt, which prevented spraying results from being as marked as in former years. However, melanose infection throughout the State for

the year was considerably less severe than is usually the case, as indicated by the relatively small percentage of very severe infection on the unsprayed parts.

TABLE 13.—*Combined results of commercial spraying for melanose control on orange and grapefruit groves at various points in Florida in 1923*

Treatment	Variety	Number of groves	Melanose infection					
			Absent	Very slight	Slight	Medium	Severe	Very severe
			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Sprayed.....	Orange.....	21	13	49	26	7	4	1
Unsprayed.....	do.....	11	0	2	23	34	35	6
Sprayed.....	Grapefruit.....	31	8	48	26	11	6	1
Unsprayed.....	do.....	14	0	2	20	40	30	8
Sprayed.....	Orange and grapefruit.....	52	10	49	26	9	5	1
Unsprayed.....	do.....	25	0	2	22	37	32	7
Homemade Bordeaux.....	do.....	38	10	53	27	7	3	0
Commercial Bordeaux.....	do.....	14	13	33	24	15	12	3
Sprayed early <sup>1</sup> .....	do.....	3	6	45	16	16	13	4
Sprayed in April <sup>2</sup> .....	do.....	45	12	49	25	9	4	1
Sprayed in May <sup>3</sup> .....	do.....	5	0	24	44	17	12	3

<sup>1</sup> Groves sprayed in February or March for citrus-scab control.

<sup>2</sup> Spraying completed before May rains set in.

<sup>3</sup> Spraying started and completed after May rains set in.

*Commercial spraying in 1924.*—Again in 1924 counts were made in groves sprayed by the owners with Bordeaux preparations for melanose control. The groves selected were representative and scattered throughout the citrus belt. They included old seedling properties as well as rather young budded groves. The results of these findings are recorded in Figures 16 and 17.

TABLE 14.—*Combined results of commercial spraying for melanose control on orange and grapefruit groves at various points in Florida in 1924*

Treatment	Variety	Number of groves	Melanose infection					
			Absent	Very slight	Slight	Medium	Severe	Very severe
			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Sprayed.....	Orange.....	18	68	19	9	3	1	0
Unsprayed.....	do.....	8	13	16	24	21	15	11
Sprayed.....	Grapefruit.....	34	68	21	9	2	0	0
Unsprayed.....	do.....	11	18	37	23	16	5	1
Sprayed.....	Orange and grapefruit.....	52	68	20	9	3	0	0
Unsprayed.....	do.....	20	16	28	23	18	10	5
Homemade Bordeaux.....	do.....	37	69	19	9	3	0	0
Commercial Bordeaux.....	do.....	15	65	22	9	3	1	0

Because of a lighter melanose outbreak than in former years, the arbitrary grading represented in Figures 16 and 17 was made more strict than formerly. Only those fruits entirely free of melanose were placed in the first grade, those very slightly and slightly blemished were placed in the second grade, and those moderately, severely, and very severely affected were placed in the third grade. On oranges the effectiveness of the spraying ranged from the maximum



control of 94 per cent to the minimum control of 17 per cent, and the best showing in the unsprayed groves was somewhat better than the least effective control by spraying. On grapefruit the sprays were very similar in effectiveness, since they ranged from a maximum control of 91 per cent to a minimum control of 33 per cent. Again the least effective spraying was not so good as the best of the unsprayed checks. The combined data from these counts are given in Table 14.

It is evident from Table 14 that melanose was not as severe as in former years. This is shown by the relatively small proportion of fruit affected severely and very severely. The control on grape-

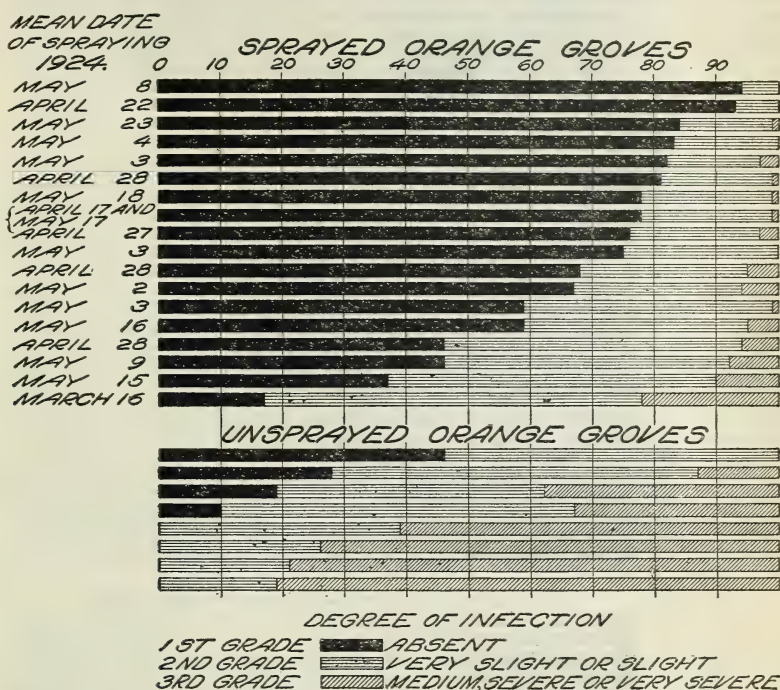


FIG. 16.—Comparison of grading of fruit with respect to melanose blemish in sprayed and unsprayed orange groves in 1924. Bordeaux-oil emulsion was used by the owners

fruit was about as good as on oranges, and commercial Bordeaux mixtures and homemade Bordeaux mixture gave practically the same results. There is not as much difference in the amount of melanose between sprayed and unsprayed fruit as has been the case in former years. This may possibly be accounted for by the fact that infection did not occur in large amounts until June, and by that time a great deal of the spray residue had probably weathered away.

There were several rainy periods in March that resulted in a considerable leaf infection, but owing to the delayed blooming period very little fruit infection occurred in that month. The rainfall was somewhat more than normal, but for the most part the rainy periods were of insufficient duration to result in general infection. May was showery, but on the average considerably drier than usual, and up to the first of June little infection had occurred. General rains set

in early in June and resulted in a rather heavy infection. It is a noteworthy fact that the general melanose-infection outbreak was several weeks later than usual, owing doubtless to the absence of prolonged rainy periods in April or May.

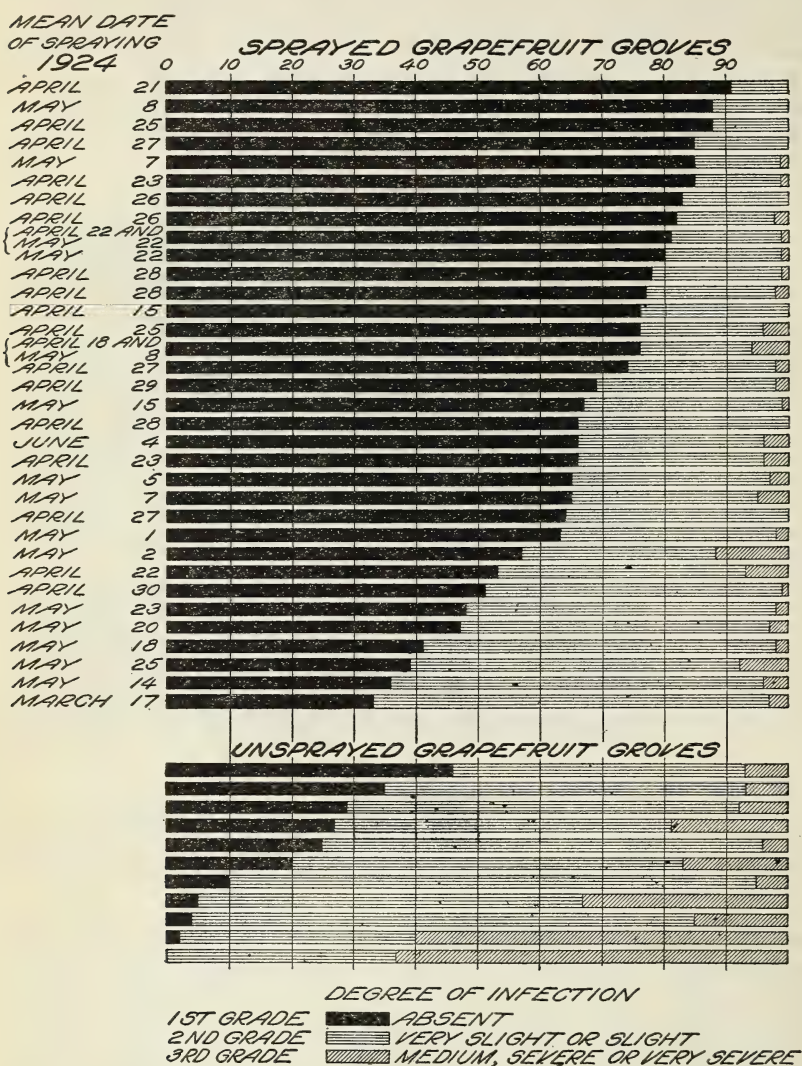


FIG. 17.—Comparison of grading of fruit with respect to melanose blemish in sprayed and unsprayed grapefruit groves in 1924. Bordeaux-oil emulsion was used by the owners

#### SECONDARY BENEFICIAL RESULTS FROM SPRAYING

It has long been recognized that Bordeaux mixture or Bordeaux-oil emulsion has a marked beneficial effect upon citrus trees suffering from certain diseases of a noninfectious nature, such as die-back and the so-called "wither tip." In addition to controlling melanose,



Bordeaux-oil emulsion applied during the spring months has a very marked beneficial effect upon chronic cases of die-back, the principal symptoms of which are "ammoniated" fruit, multiple buds, gum pockets, bark excrescences, and stained terminals.

In groves that annually produced crops of poor quality, due to "ammoniation," prompt and almost complete relief to the current crop has been observed repeatedly when Bordeaux-oil emulsion was applied during the spring months. This is particularly true of old seedling orange groves in flatwoods soils and where foot rot is accompanied by "ammoniation."

Frequently orange and grapefruit trees that are in a run-down condition with symptoms of wither tip due to obscure causes resume normal growth following an application or two of Bordeaux-oil emulsion made during the melanose spraying season.

#### COST OF SPRAYING

The cost of spraying is difficult to arrive at, but on the average a power outfit with a team and three men seldom applies more than 1,600 gallons a day, frequently 1,200 gallons or less. Spray materials should not cost more than  $1\frac{1}{2}$  cents a gallon for Bordeaux-oil emulsion. An average-sized mature orange or grapefruit tree that produces from 3 to 4 boxes of fruit annually requires from 8 to 10 gallons of spray to wet all parts thoroughly.

Since the cost of spray materials in Bordeaux-oil emulsion represents about 50 to 60 per cent of the entire cost of spraying, the gross cost of spraying should not be more than 30 cents a tree or more than 10 cents a box, and frequently it is less than 8 cents a box when all costs are considered. The total annual cost of all necessary fungicidal and insecticidal applications for the average citrus grove in Florida oftentimes does not amount to more than 15 cents a box of fruit. Although spraying is a slower operation than dusting, it is not likely to be more expensive.

#### VALUE OF SPRAYING

To sum up, in the light of present knowledge, spraying is the only means of controlling melanose that is economical and effective for use in normal mature groves. Sulphur sprays have little effect upon the disease, the weaker copper sprays are only partially effective, but standard 3-3-50 Bordeaux plus 1 per cent oil as emulsion applied before much infection takes place gives an excellent control of the disease.

#### SPRAY INJURY

Generally speaking, citrus fruits are much more resistant to spray injury than are peaches or apples. Nevertheless, all of the principal types of sprays used in citrus groves are capable of producing distinctive forms of injury.

*Copper-spray injury.*—The orange fruit or foliage is seldom injured by a reasonable number of properly made applications of Bordeaux mixture or Bordeaux-oil emulsion. When injury does occur on the orange the trouble is likely to be due to the water used in spraying, or to poor spreading of the spray, or in rare instances to

insufficient lime in the Bordeaux mixture. Spray burn of the fruit of the orange has rarely occurred on the experimental plots except where an excessive number of applications were made, and in only a few instances has it been reported by commercial growers.

Although occurring in small proportions, a peculiar type of rind blemish developed on the oranges that received many applications of Bordeaux-oil emulsion (pl. 6, D), and it was not observed on unsprayed fruit. This has been observed several times and is considered to be spray injury. It has not been observed on grapefruit.

The excessively sprayed oranges colored up about two weeks earlier than the unsprayed checks, and they also took on a deep orange, almost tangerine, color; the taste became insipid, the rind coarse and puffy; creasing developed before Christmas; and the fruit dried out three or four weeks in advance of unsprayed fruit.

Similar conditions have since been observed repeatedly where oranges were sprayed several times with Bordeaux-oil emulsion during the rainy season or where bluestone was applied to the ground around the trees during that season.

Still another unusual condition developed only on orange trees in the form of what is usually called "wither tip." A number of twigs died back during the early or middle fall, and moderate but general defoliation occurred during early winter for no apparent reason. This condition has also been observed repeatedly on trees sprayed with Bordeaux-oil during the summer months, but not on unsprayed trees.

Star melanose (pl. 7, D), described on page 3, occurs on leaves and fruit of both the orange and the grapefruit. It, too, is doubtless a form of Bordeaux injury, as it has been observed repeatedly on trees sprayed excessively with simple Bordeaux mixture as well as on those sprayed with Bordeaux-oil emulsion. This blemish has never been observed on unsprayed trees.

There is still another type of spray injury that sometimes develops on leaves of orange and grapefruit sprayed with Bordeaux-oil emulsion. This type of spray injury (pl. 7, A, B) is of rather rare occurrence and manifests itself as rather large raised areas on either surface of the leaf.

*Oil-spray injury.*—Young orange and grapefruit trees sprayed with oil emulsion in late spring or early summer during the melanose spraying season are likely to develop immediately signs of oil injury in the form of what appear to be oil-soaked areas commonly called "shadows" on the cheeks of the fruit. This is undoubtedly the most common form of oil injury, and, except in severe cases, it disappears before the fruit ripens. By the addition of standard Bordeaux mixture to oil emulsion applied before the onset of the heavy rains, melanose can be prevented and oil shadows avoided.

Another type of oil burn met with in grove spraying is found on the lower side of the fruit as solitary depressions of rather regular margin where drops of oil spray have collected and dried. This injury occurs on both orange and grapefruit, and is usually of minor importance except when the oil emulsion breaks.

The most usual type of Bordeaux-oil spray burn occasionally found on the grapefruit and very rarely on the orange is illustrated in Plate 6, C. This blemish develops only on the sunny side of a



fruit, and is found for the most part on the outside of the tree where the fruit is exposed to the sun. The surface tissue is killed and turns brown. Later it may slough off, leaving only a slight blemish, but usually the injury reduces the grade of the affected fruit. Recent developments indicate that the occurrence of this blemish may be reduced by the use of calcium caseinate as a spreader at the rate of about 1 pound to each 100 gallons of spray.

*Sulphur-spray injury.*—Sulphur sprays when applied in exceedingly hot weather, especially when in combination with oil emulsion, may be the cause of some fruit injury. This type of injury occurs usually as a large spot with an irregular margin, invariably on the sunny side of the fruit. The affected area may be killed for a quarter of an inch or more in depth; the surface becomes grayish, and later the dead tissue is likely to crack. (Pl. 6, E.) This type of injury frequently develops when an application of oil spray is made during the summer within a week or 10 days after an application of sulphur dust or spray. Applications made in the reverse order seem to be about equally dangerous. Combination sulphur-oil sprays are also very dangerous when applied in hot weather.

#### SPRAY SCHEDULE

Table 15 presents a spray schedule for melanose control which is intended to be used only when the spring is unusually wet and conducive to melanose infection. In average seasons one application made between April 15 and May 5 with 3-3-50 Bordeaux plus 1 per cent oil as emulsion gives satisfactory melanose control. Application A is timed to fall in the last of the scab-spraying periods, and it would therefore serve a dual purpose on those forms that are susceptible to scab as well as to melanose. It is, however, too early for best results against melanose, and therefore a second application about a month later is sometimes a paying investment, but it may be omitted if May is dry. Under average conditions only one application is needed. The most effective time for this is just in advance of the heavy infection, which seldom occurs before May 5 (application B).

TABLE 15.—*Spray schedule for the control of melanose in rainy seasons*

*Caution.*—Spray with oil emulsion in late June or early July to control scale insects. Do not fail to control rust mites, otherwise the prevention of melanose is of little value because of the later russet injury. (26.)

Application	Time <sup>1</sup>	Material	Object
A.....	Apr. 1 to 10...	3-3-50 Bordeaux mixture plus 1 per cent oil, as oil emulsion. <sup>2</sup>	To protect young fruit against melanose; also effective against late citrus-scab infection.
B <sup>3</sup> .....	May 1 to 5....	do.....	To protect young fruit against melanose.

<sup>1</sup> In seasons of normal rainfall one application made between Apr. 15 and May 5 gives satisfactory melanose control.

<sup>2</sup> Three gallons of oil emulsion (Government formula or its equivalent) in 200 gallons of Bordeaux mixture gives 1 per cent of oil.

<sup>3</sup> This application of Bordeaux-oil emulsion will take the place of the usual white-fly spraying.

The 3-3-50 Bordeaux mixture may be made in the usually recommended way, from stock solutions of bluestone and of quicklime. A more rapid and entirely satisfactory method now in general use in

Florida is to place the requisite quantity (12 pounds for 200 gallons of spray) of finely pulverized bluestone in a suitable strainer in the opening of the spray tank. This will dissolve as the tank is filled. When nearly full, start the agitator and sift in 16 pounds of builders' hydrated lime (taking the place of 12 pounds of quicklime usually specified). Last of all add 3 gallons of oil emulsion while the agitator is kept running.

#### EXTRA SPRAYS FOLLOWING MELANOSE APPLICATION

Spraying with any material that will effectively prevent melanose infection will very likely be about equally disastrous to the fungi parasitic upon citrus insects, thereby resulting in a considerable and usually rapid increase in scale insects. Experience has shown that wherever simple copper sprays are applied, or even combinations of such sprays with oil emulsion, during spring months, a follow-up spray of oil emulsion should be made to prevent serious damage from these pests. The Bordeaux-oil emulsion in April or early May is not only effective against melanose but also controls the white flies as well. Nevertheless when this spray is applied an oil emulsion of high efficiency should be applied in late June or early July while the scales are in an immature state. Sometimes this extra oil spray may be delayed until fall, but such delay is not safe and may lead to considerable damage from scale insects during the summer.

#### SUMMARY

Melanose, a fungus disease caused by *Phomopsis citri* Fawcett, attacks a wide range of rutaceous plants including all citrus forms grown commercially in Florida. This organism is also the cause of a stem-end rot that occurs in large proportions on Florida citrus fruits.

*Phomopsis citri* sporulates in nature only in dead bark of twigs and limbs; it has never been observed in or cultured from melanose lesions on living parts. Spore production, germination, infection, and incubation are markedly retarded by temperatures either above or below the apparently optimum range of from 68° to 81.5° F. (20° to 27.5° C.). Infection may take place at any time of the year when rainy periods occur while growing parts are in a susceptible stage.

Leaves of the orange and grapefruit are susceptible to infection from the time they emerge from the bud until they become distinctly tough; this usually requires about two or three weeks. The fruits of the orange and grapefruit are at first very susceptible to infection. They become progressively resistant with increasing size; oranges develop practical immunity when they reach about 1½ inches in diameter, whereas grapefruits do not become practically immune until they reach about 2½ inches in diameter.

Under Florida conditions, oranges and grapefruits from February or March bloom become practically immune in May; seldom are they susceptible to infection after June 10.

Pruning away the dead and weakened parts, thereby removing the source of infection, has not proved to be a dependable means of preventing melanose outbreaks under ordinary commercial condi-



tions. Severe pruning or deheading such as is practiced after damaging freezes usually gives a good control of the disease provided the pruning is done before the May rains set in.

Fungicides applied as dusts, such as sulphur preparations, Bordeaux dust, and copper-lime dusts, as a rule have not given satisfactory control of melanose. It seems that no reasonable number of applications of the present-day dusts will insure a crop of fruit commercially free of melanose.

Sulphur sprays and the weaker copper sprays have failed repeatedly to control melanose.

A single application of the standard 3-3-50 Bordeaux mixture plus 1 per cent oil as emulsion, if properly timed, gives excellent control of the disease even under adverse conditions. Ordinarily the most opportune time for this application is just in advance of the May rains, which seldom set in before the 5th of the month. If the spraying is delayed until after these rains have continued for some time, melanose prevention is not obtained. Sprays applied even before the blossoms open may reduce the chances for fruit infection somewhat; those applied early in April give better protection to the fruit; and those applied in late April or early May, before the rains set in, insure a still better protection.

Whenever applications of Bordeaux-oil emulsion are made in the spring they should by all means be followed in late June or early July by a thorough application of oil emulsion of high efficiency to prevent excessive increases in scale insects. If this necessary oil spray is omitted the fruit is likely to be ruined and the tree seriously damaged by these insects.

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